U.S. ENVIRONMENTAL PROTECTION AGENCY (EPA)

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FEDERAL INSECTICIDE, FUNGICIDE AND RODENTICIDE ACT SCIENTIFIC ADVISORY PANEL (FIFRA SAP)

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REEVALUATION OF THE HUMAN HEALTH EFFECTS OF ATRAZINE: REVIEW OF EXPERIMENTAL ANIMAL AND IN VITRO STUDIES AND DRINKING WATER MONITORING FREQUENCY

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The Panel convened at 8:30 a.m. in the Hamilton Ballroom of the Hamilton Crowne Plaza Hotel, located at 1001 14th Street, N.W., Washington, D.C., Steven G. Heeringa, Ph.D., Chair, and Kenneth M. Portier, Ph.D., Session Chair, presiding.

FIFRA SAP MEMBERS PRESENT:
STEVEN G. HEERINGA, Ph.D., Chair
KENNETH M. PORTIER, Ph.D., Session Chair
JOHN R. BUCHER, Ph.D., DABT
JANICE E. CHAMBERS, Ph.D., DABT, ATS
GERALD A. LeBLANC, Ph.D.
DANIEL SCHLENK, Ph.D.

FQPA SCIENCE REVIEW BOARD MEMBERS PRESENT:

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RICHARD H. COUPE, Ph.D.

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TERESA H. HORTON, Ph.D.

KANNAN KRISHNAN, Ph.D.

HERBERT K.H. LEE, Ph.D.

KEVIN T. O'BYRNE, Ph.D.

NU-MAY RUBY REED, Ph.D., DABT

JEAN F.L. REGAL, Ph.D.

DANIEL J. SELVAGE, Ph.D.

CARMEN J. WILLIAMS, M.D., Ph.D.

LINDA J. YOUNG, Ph.D.

ALSO PRESENT:

JOSEPH E. BAILEY, Designated Federal Official

TABLE OF CONTENTS

P-R-O-C-E-E-D-I-N-G-S

8:30 a.m.

MR. BAILEY: We've got a lot of material to cover over the next two days, and we're anxious to get going. I'm Joe Bailey. I'm serving as the DFO for the meeting, Designated Federal Official.

I just wanted to let the panel know that I tried to e-mail the public comments and presentations last night, but some of the e-mails kicked back because they were too big. What a surprise. But I will make a second attempt this afternoon or tonight to get it to everybody, so everybody has all of the presentations electronically.

At this point I'll turn it over to Dr. Portier, our Chair for this meeting. Thank you.

DR. PORTIER: Good morning. I'm Ken Portier, Chair. This is the -- I guess it's the third day of the FIFRA Scientific Advisory Panel meeting on the re-evaluation of

the human health effects of atrazine, review of experimental animals and in vitro studies and drinking water monitoring frequency.

We've twice introduced the panel, so I don't think at this point we need to reintroduce the panel. Most everyone in the audience is probably familiar with us now. And we have before us the bulk of our task, we have remaining 12 questions, and two days to do it, 16 hours. So my goal is to average about 50 minutes a session, take a break after two questions, and break at five o'clock this evening. All right. So we're not going to push it like we did yesterday. I realize people have kids -- staff have kids to pick up and -- yes, it's easy to keep people happy. Right?

It's unlikely we're going to get to any of the hydrology questions today, which gives that part of the panel time to really delve into that a little bit more. My goal will be to get eight questions done today, 1.2

to 1.9. I'll be happy if we do that, but I -- you know, even if we just get through 1.8 I think we'll be doing well.

So with that I will ask Dr. Mendez or Dr. Lowit, if they have any opening comments from EPA, or Dr. Cooper?

DR. MENDEZ: We don't have any opening remarks at this time. And Dr. Cooper will start reading Charge Question 1.2.

DR. PORTIER: Thank you.

DR. COOPER: I didn't know I was going to do this. So the Charge Question 1.2 -- in Charge Question 1.2, based on an evaluation of the studies examining the mode of action at atrazine on neuroendocrine function, Section 3.2 of the draft issue paper, the Agency has preliminarily concluded that atrazine affects both the HPG axis and the HPA axis.

With respect to the temporal concordance, recent studies show that atrazine induces a rapid, within minutes, increase in

ACTH and adrenal cortical hormones, corticosterone and progesterone, in both male and female rats, Fraites, et al., 2009; Laws, et al., 2009; and Pruett, et al., 2009, while changes in the HPG axis, such as suppression of the LH surge, may take up to two to three days, Cooper, et al., 2009. That should be 2000, by the way.

(Laughter.)

DR. COOPER: Note the mistake in that, please, so I'm not on the griddle to produce a paper last year.

This sequence of HPA alterations followed by HPG changes indicates that the suppression of the surge may be, in part, mediated by activation of the HPA axis, i.e. a corticosterone suppression of the GnRH and LH release. In addition, with respect to the dose-response concordance, atrazine-induced increases in ACTH, corticosterone and adrenal progesterone are seen following a single dose of atrazine, 50 milligrams per kilogram.

In contrast, the HPG is not altered by following a single dose up to 200 milligrams per kilogram. However, when treated for multiple days, the dose necessary to alter the HPA axis is lower than or equal to the one needed to affect the HPG axis.

Charge Question 1.2, please comment on the Agency's preliminary hypothesis for the mode of action involving atrazine's alteration of both the HPA and the HPG axes. Does the document adequately and clearly describe the hypothesis in the context of a toxicity pathway, i.e. cellular responses that, when sufficiently perturbed, are expected to result in adverse health effects? Does the document clearly describe the data used to test the proposed hypotheses? To what extent do the available data establish key events in the proposed mode of action hypothesis? And what are the strengths and limitations of the data available on this hypothesis?

Please include in your comments a discussion of the Agency's interpretation of the data linking the initial perturbations in the HPA axis to the changes in the HPG axis.

Is there more?

DR. MENDEZ: Yes, there's more. Hold on.

DR. COOPER: Long question. Please comment on the evidence that the initial perturbations in the HPA axis may lead to impairments in reproductive function and/or developmental consequences. Which events is/are viewed as critical in leading to health consequences? Are there data on other substances that would inform this question?

DR. PORTIER: Thank you. We've kind of spread out the panel a little bit more.

So, Dr. O'Byrne, you've got a little of room. You ready to kick us off here?

DR. O'BYRNE: Thank you. Thank

you very much.

I think the EPA's preliminary hypothesis for activation of atrazine's mode of activation -- mode of action involving alteration of both the HPA and the HPG axes is adequately described and scientifically sound based on the experimental data that's available.

The details of the toxicity pathway, however, is very, very limited, and the mechanisms of action of atrazine on the perturbation of the HPG I'll describe in a minute. The mechanisms of action on the HPA perhaps should be moved into questions 1.3 and 1.4 because there's some overlap there.

But the key question of the causal link between the activation of the HPA and the subsequent suppression of the HPG axis is far, far from conclusive. Nevertheless, I think the available data provides a reasonable case for the Agency to put forward that preliminary hypothesis.

Now I don't wish to review the post data, post-2003 data on the HPA and HPG axes. I'm very mindful of the Chair's comment at the beginning of this that we shouldn't repeat what other people have said or what we say ourselves, otherwise we'll be chopped off in mid-sentence. So that's a great advantage to me.

(Laughter.)

DR. O'BYRNE: But nevertheless, I think Dr. Handa's additional information yesterday was particularly helpful. And of course you'll remember that. I would like to make a very brief comment about the interaction between the HPA and HPG axes, just in case there are people in the audience that are new.

It is extremely well-established that there's an inverse relationship between the HPA and the HPG axes, which has led to the hypothesis that activation of the HPA results in a suppression of the HPG. This is not

rocket science, and this is particularly true in response to stressful stimuli. And in clinical scenarios as well. For example, in Cushing's disease there is a beautiful inverse relationship between circulating levels of cortisol and the degree of reproductive suppression in terms of normal menstrual cycle in women, oligo, and complete amenorrhea.

So this physiological correlate of the inverse relationship, coupled with the new evidence that atrazine activates the HP axis, has obviously led the Agency to this preliminary hypothesis that the mode of action of atrazine involves an alteration of both of these critical axes. And the influence is sequential rather than some parallel fashion, so that changes in the adrenal steroid hormones are driving the deleterious effects on the reproductive axis. So I just wanted to give you that brief summary.

Now my major comments relating to atrazine. There's clear evidence that there's

a transient increase in corticosterone, and we've seen evidence of that with rapid increase and return to baseline within six hours. Whether this is measured after a single injection, or exposure, or after a 28-day exposure and the final administration results in an indistinguishable rise and fall in corticosterone.

Now there's ample evidence that increases in -- acute increases in corticosterone, cortisol depending on the species, do not, and I mean do not affect the pulsatile release of LH. And this is in a huge range of species, from rats all the way up to humans. There is, however, one exception and that is the sheep. And here, physiological stress levels of cortisol, or cort, results in a rapid decrease in LH pulse amplitude. And this happens within 30 to 60 minutes.

And this is a pituitary phenomenon, where there's a reduction in

pituitary sensitivity to GnRH, the tropic stimulus. But there is very clear, clear evidence that atrazine does not have this pituitary effect, and I think that data is quite solid and unambiguous. And in the sheep, incidentally, it also has an acute effect on pulse frequency, and we don't really need to go into that.

Now, in contrast to these -- the lack of evidence of an acute effect of corticol, corticosterone, on LH pulse frequency, evidence concerning the effects of acute stress, or cort, on the surge release of LH is less consistent. However, chronic levels of these hormones, their effects on the surge generating mechanism are quite consistent.

And I'd like to just talk to you a little bit about the data that is available. And in the context of the rat, it's actually pretty meager. But, nevertheless, back in the late `70s, Baldwin and his colleagues showed

that a single injection of dexamethasone or cortisol at two to eight hours after the administration of estradiol benzoate did not block or affect the LH surge in rats. So that's fairly conclusive that acute increases in glucocorticoids do not suppress the LH surge in this species.

However, if he takes a chronic approach and implants a capsule where you get chronic elevated levels of cortisol, then you disturb the cycles for weeks and you completely block the pre-ovalutory -- the spontaneous pre-ovalutory gonadotropin surge. So clearly chronic is a good example of chronic cortisol having an effect.

Then in the mid `90s there was another study where somebody restrained rats for various time points, zero, one, and two hours before the estimated time of the onset of the spontaneous surge, and continued that restraint until lights went out, which basically means after the surge is over. And

in those rats 50 percent had no surge and 50 percent had an attenuation, or a decrease in amplitude. So here we have two rat studies that actually conflict in some ways. But you can sit here and debate the reasons why that may be the case.

I'd like to describe just a couple of experiments that have been carried out in the sheep, and there's been a very interesting flurry of activity very, very recently in the sheep, which has been initiated by one of your great physiologists, Fred Cosh, who actually should be here rather than me. Perhaps Joe was less convincing and persuasive with Fred Cosh. I don't know. But, well, maybe he's enjoying his retirement.

But he really has spurred the sheep people interaction around the globe. And, in particular, Harry Dobson in Liverpool in the UK, and what she's done is that she's acutely activated the HP axis in her ewes with insulin-induced hypoglycemia. And she's done

this at 30 and 32 hours after withdrawal of progesterone. What the sheep guys do is they have their sheep, they put an implant of progesterone in and then they pull it out, and then 48 hours later you have a spontaneous LH surge. And this is synchronizing the surges so to -- it facilitates their experimental design.

So she induced hypoglycemia, and very close to the surge. It decreased circulating levels of estrogen significantly, but not markedly. And this resulted in a delay of the LH surge by nine hours. What Fred Cosh has done, and this was published this year, Fred Cosh has also done an experiment, but instead of using a single acute stress, he's used a series of acute stresses, and these are psychological stresses. And he's exposed his ewes to thee psychological stresses at 12 to 18 hours, 24 to 30 hours, and then 36 to 42 hours after that progesterone implant is pulled out.

Remember, the surge occurs at 48 hours.

And that repeated acute stress paradigm, and he had a whole range of these, I've just described one of them, had absolutely no effect on the LH surge. It occurred on time, normal amplitude. So here we have two sheep people had papers published this year that also are very, very different.

But what Fred did is, if he administered physiological levels of cort, corticosterone, for 42 hours, then he delayed the LH surge by 10 hours, just like Harry Dobson. And there's also a reduction in amplitude. But you've got to remember that the sheep is quite unusual in this context of amplitude modulation in response to physiological levels of cort.

I think I'd also like to remind the panel that there isn't a shred of evidence that the GnRH surge generating mechanism in the rat, or indeed the sheep, bears any resemblance to a human or primate, and I think

that's actually very important. And we can discuss that if people think that's necessary.

So how does atrazine fit into all of this? Well, although there is clear evidence that sustained or chronic levels of glucocorticoids suppress the pulse generator, and I hope that's fairly clear, in a wide range of species, from rats right up to humans, there is a lack of evidence that atrazine causes a sustained increase in corticosterone.

Indeed, if you look at the data that was presented in the last couple of days from Pruett published in 2009, daily exposures to astronomical levels of atrazine, we're talking about 150 milligrams per kilogram per day given intraperitoneally, resulted in a transient increase in cort that was no different from that from a single injection. So up and down within six hours.

Dr. Handa showed us yesterday that 50 milligrams per kilogram increased

corticosterone for less than an hour in the rat. The other study was in the mouse. So the notion that a three-day protocol of exposure to 50 milligrams could result in a complete blockade of the LH surge, I think is unlikely. And then the sort of clinch was yesterday again, when Dr. Handa showed us that adrenalectomy didn't block the surge induced -- the blockade of the surge induced by atrazine. So I think that really does make one appreciate that the glucocorticoids perhaps are not involved.

With respect to the health consequences, it's my considered opinion that there is little evidence, perhaps no evidence, of adversity because the concentrations that have been used in the various experiments that I've reviewed draw no comparison whatsoever to what we would expect humans, or indeed wildlife, to be exposed to.

This may -- it may be very different for amphibians. I mean these guys

live in the water. They're like a blancmange; they absorb everything through their skin, and very readily. And I think this was elegantly portrayed yesterday by Pastoor's comments. That's my opinion.

I think there is a dire need for new data, particularly experiments involving environmentally relevant doses of atrazine, given chronically, via an oral route, and I don't mean by gavage, I mean in their drinking water. And it astonishes me and some of my other colleagues here that nobody's actually looked at the effect of such administration of low levels that one would expect animals to be exposed to, and looking at the effects on corticosterone release, basal corticosterone release. It's not been done, and that is absolutely mind blowing. That's the first experiment that I would have done, and in those same animals you could have looked at various physiological reproductive parameters.

And I think the comment that Dr.

Gilliom made yesterday about concurrence is very, very well taken. And he simply added to the bottle of water. So that's easy. Now Dr. Mendez described key additional experiments and data that's going to come out in spring and summer of 2010. Well, spring's here and it feels like summer's here as well, to me, coming from London. But, you know, some of those are critical and I'm wondering when they're going to come.

But I cannot impress the importance of looking at brain expression of critical neuropeptides. I mean the fact that nobody's bothered to look at CRF and vasopressin expression is again astonishing. Looking at GnRH levels by microdialysis is something that I think should be done, and there's somebody sitting around this table who is an expert in this, and this is not a trivial technique, as very few people can do it properly. And you've got somebody in your presence who can do this beautifully. So

people should take advantage of that.

And the catecholamines. I mean one can't escape from those. And then kisspeptin, I mean Dr. Mendez mentioned this in her slides. Kisspeptin's been around for something like seven years. It's the new thing on the block in terms of for the reproductive physiologists, and it's the driving force to the GnRH neurons. And, again, nobody's bothered, as far as we can tell, to look at the expression of kisspeptin in response to this herbicide. And these are very simple and easy experiments to do, and would give us a huge amount of information that would make us -- make it easier for us to give an informed decision.

So I think that's all I wish to comment in my response.

DR. PORTIER: Thank you.

Dr. Bucher.

DR. BUCHER: Thanks.

John Bucher. So, in general, I

thought that the document adequately established the interrelationships between disruption of the HPG axis and the resulting phenotypic effects including reproduction, development, immune endpoints, and cancer in experimental animals. There's a general consistency in dose, response, and timing that are required to satisfy a mode of action, and the document does a reasonable job of interpreting the recent literature in the context of the proposed MOA.

In my opinion, more could have been said about the biological plausibility and support for cross-species applicability that the literature on the HPA axis and stress research in general that you just heard about brings to the proposed MOA. Although I'm not an expert at all in this area, the human relevance of using disruption of the LH surge and the benchmark dose calculation is, in my mind, strengthened by the extensive documentation of effects of various types of

stress acting through the HPG axis to affect human reproductive health.

That said, I don't believe that figure 3, which is the schematic of the proposed MOA on page 30 of the white paper, does a good job of capturing the key events in the proposed MOA. The problem is that it goes into too much detail in trying to describe what are, in essence, redundant mechanisms that are typically involved maintaining homeostasis.

This also relates to the question of whether there's a clear toxicity pathway described in the document. Although Dr. Cooper, on Monday, said he was leaning towards the right side of the house through the HPA axis as representing a direct or possibly a linear pathway for atrazine's biological effects.

I don't think that these highly dynamic mechanisms really lend themselves to the toxicity pathway concept. For example, LH

secretion is directly sensitive to gonadotropin-releasing hormone, but also indirectly to gonadotropin-inhibiting hormone, ACTH, corticosterone, corticotropin-releasing hormone, and possibly progesterone.

It is not clear why, to me yet, why the data on temporal relationships between the stimulation of the HPA axis and the disruption of the HPG axis are what they are. I think it may be more useful to scale back your expectations for making all of the data fit within a coherent framework. Toxicity pathways most likely are not linear, but rather are webs with nodes that represent convergent points, or points of greatest sensitivity. Figure 3, to me, is really a web with LH pulse suppression as a sensitive node.

Despite this, I think the Agency's on solid ground in using disruption of the LH release and other biological endpoints that have been directly associated with it as measurable key events. This endpoint is the

most sensitive in terms of the atrazine chronic dosing studies and it's been plausibly linked to the phenotypic outcomes of interest.

As with the application of other MOAs to specific situations, if you look closely at how well the MOA fits specific data sets, you'll see weaknesses in fits to the model. But as we've all heard many times, all models are wrong, but some are useful. I think in this case the disruption of the HPG axis is useful and the Agency should move forward with it as a point of departure until something clearly better comes along.

That concludes my comments.

DR. PORTIER: Thank you.

Dr. Akana?

DR. AKANA: What I'll do is just add what has not been covered already, and I concur with quite a number of the points. In my opinion, the hypothesis, like many, is a maturing hypothesis, and I don't think it's quite developed to the point where it should

be aggressively pursued at this point.

Now what do I mean? One of the points of the hypothesis is that there is a central action of atrazine, and here is where I think some important spotty sets of data are missing. And when we talk about central action, we're not talking about the brain, and the type of evidence we've had so far is, again, a little bit lighter on what you find in the brain.

I think it's very promising that we heard some of the cFos data and some of the gonadotropin -- excuse me, the GnRH brain data, but I think it's absolutely critical that in the same kinds of animals they also look at CRF and ABP in those brain sections. And if they also layer in there with cFos, you can get some sort of temporal sense of which one is reacting first. So that part, I think, is critical.

Now when we talk about the activation of HPA system, there's the

hypothalamus, so that is relatively untouched. The data on pituitary, POMC type of production, that's also very lightly touched. But what most concerns me when you talk about activation of the HPA system; it is a feedback system, a negative feedback system. And the very preliminary data that we've have, which is really exciting, that Dr. Handa talked about with the adrenalectomy, is the direction I think the studies need to go.

What we've seen are some really lovely datasets on characterizing the response to the HPA to atrazine in terms of ACTH and corticosterone. And the data sets are strongest with a single injection, a single acute injection. The time frame and the dose response and the time domains are very nice for saying that atrazine stimulates the HPA system with a single acute injection.

Now, the other fun thing about the HPA system is with one of the outputs, just one of them, which is corticosterone. As we

all remember, corticosterone is also a glucocorticoid, and that is a hidden partner in the hypothesis that must be addressed. Because it's a glucocorticoid, you're changing metabolism. Okay. In the framework of an acute response, that is actually not a major, major concern.

But as soon as you move into a chronic model, a chronic model of atrazine, you enter a whole new type of dynamic. What has been emerging in the last 10 years is in the stress system, when you move into repeated or chronic stress, you have a different brain. Okay. CRF and PVN, that one is pretty well recognized, but CRF is found in other cell bodies, cell brain nuclides in the brain. It's a distributed system, it's responsive to stress, they interact. There are neural connections that are proven, and they have major, major outputs.

So you have CRF and PVN, but you also have it in BNST, in the central nucleus

of amygdala, you have in the prefrontal cortex. And when it's activated in a stress circuit, what you get is actually positive feedback of corticosterone onto those systems, and it drives other behaviors. So you have a different kind of animal, which -- with chronic stress, and it might help make some of the data in the repeated atrazine application much more coherent when you are sensitive to that.

Now when we talk about glucocorticoids and the metabolism, this is one of my areas of energy balance, you are feeding into the reproductive system. The reproductive system is sensitive to energy substrate flows. So I would urge a more -- a larger picture taken with the data, that you look not only on the HPA and the LH surge, but in the framework of what kind of energy peak the animal is. For instance, atrazine very often reduces body weight.

And now, in terms of the animal

and negative energy balance, this is critical how it nuances some of the LH surge and also the HPA. So what I'm saying is, I think the hypothesis needs to evolve to address the acute response somewhat separately from the chronic response.

That was my major point. I also would like to table the toxicity pathway to the next charge question. I think the key events are still somewhat murky, therefore, in this change because we don't know precisely what's happening, the atrazine action, as it kicks off in the hypothalamus.

Now the idea that it works primarily on the HPA is perhaps conditioned, because the literature shows there is, again, a very strong interaction in the chronic state between the HPA and the HPG. And there's some very nice models of where HPA and HPG axes are rendered independent of each other by controlling both the corticosterone level and, in the male rat, the testosterone level.

And here I'm thinking of a model of Victor Viau, and I think that is a fabulous template to consider how to interpret atrazine action when you have independent control of each axis. So overall, I think the strength of the hypothesis is mostly in acute injection -- in acute atrazine exposure. I think it's much more limited in the chronic state, and I think there are many tools that can -- that are in place already and well-established that can be used to tackle those problems, and I would urge the panel to encourage those be employed.

And I'll stop there. Thank you.

DR. PORTIER: Thank you.

Dr. Fenner-Crisp?

DR. FENNER-CRISP: Given what's already been said, I don't want to repeat any of that. I support most of the comments made. I'd like to focus mine on exposure root and its relevance in dose concordance. I can appreciate that in the laboratory setting one

wants to do experiments that see something, and that generally can explain why higher doses are used, at least in the first round. But I also would appreciate, as Dr. O'Bryne's comment, one wants to ultimately understand what may or may not be happening at human exposure relevant levels.

The body of literature in vivo show studies by several routes, oral gavage, some sub-Q, some IP, and one would have to try to understand what difference does that make in the results in comparing them across those studies with respect to pharmacokinetics. Would you be seeing different patterns of percentages of rate and percentages of metabolic formation, and conjugation, the whole ADME scheme, depending upon the route-specific application?

And how does that affect the results that one sees and how does one take that into account when trying to do a dose concordance across these varying routes of

exposure, trying to compare and understand their relationship, particularly when trying to understand where, how, if the HPA axis disturbances impinge on the HPG.

The Agency mentioned somewhere in the white paper that they plan to do benchmark dose analysis somewhere along the way when they get to the hazard characterization. It may be useful to do that sooner rather than later to get a better understanding of dose concordance in hazard identification.

And I am assuming that one's going to apply somebody's, not the Agency's, pre-existing PBPK model to expand on that understanding. That would be a recommendation that I would offer.

I think that it may come to the point where some of the studies that have been done, and offer some kind of results, may have to be redone by more relevant routes of exposure to confirm or not those particular findings, if at a point in time they are

thought of as being critical to the understanding of the pathways, and an integral part in doing the risk assessment.

DR. PORTIER: Thank you.

DR. SELVAGE: Okay. Thanks.

My response is -- it kind of segues into the next question as well, so I'll keep this part pretty limited. I agree with most of what's been said. I think Dr. O'Byrne did a really nice job of describing, you know, a lot of concerns that I think are valid. You know, I guess I'll just add that, you know, I do think the document does describe the hypothesis well; I think that's clear.

However, I don't think there's nearly enough data yet to, you know, draw the conclusion that the EPA is leaning towards, in that it's the HPA axis disruption that's affecting the HPG function. You know, I would say that, you know, in thinking about this from a CNS perspective, you have -- there's a lot of overlap between the HPA and the HPG

axis at the level of the CNS in terms of, you know, receptors for gonadal hormones being present in areas that are going to affect HPA axis function, and vice versa. So I think, you know, it's going to be important to look at that.

Also, a lot of stimuli that are going to affect the HPA axis are also going to affect the HPG axis is what I'm trying to get at here, so, you know, perturbations of a lot of neurotransmitter systems that are going to affect the HPA axis are also going to affect the HPG axis, you know, and they might be doing this -- affecting each axis separately. So I think, you know, you could be having some other central action affecting both of these systems. So I think it's really important to look at what's going on at the level of the brain in much more depth.

Also, I need to concur with Dr. O'Bryne on the need for more cort measurements, basal cort measurements, in

particular, and to my mind this is especially important in the chronic studies because all the cort measurements that we've seen have been after atrazine in the chronic studies. You know, there's certainly a possibility that, for one thing, the circadian rhythm of cort could possibly be disrupted by atrazine.

Also, you know, it seems possible to me that you have up-regulation of the HPA axis in general in these females. It's certainly a possibility. I think that needs to be tested, for instance, give atrazine, you know, chronically then stop the atrazine and on the next day give another stressor and see how their response compares to, you know, animals that haven't received atrazine, I think, if you want to see if you're getting an up-regulation in general of the HPA axis.

I think there's, you know, some possibilities with, you know, the proposed mechanisms of action, for instance the phosphodiesterase inhibitors, the increased

cyclic AMP, and cyclic AMP for instance is -- cyclic AMP pathways are involved in the -- stimulating the transcription of CRF. So I think looking at those areas will be important.

Other than that, I think a lot of this will just feed into the next question. You know, I think there's a good -- you have a good starting point, but I think a lot more needs to be done to draw a firm conclusion. Right now the conclusions are being based on correlations, and obviously correlation doesn't mean causation. So I think at that point we could probably go on to the next question and let -- well, after other comment.

DR. PORTIER: Thank you. At this point we'll open it up to comments from anyone on the panel.

Dr. Krishnan. Was it -- oh, Dr. Akana.

DR. AKANA: I wanted to reinforce a point that Daniel made, is that in a lot of

the timing of samples taken, it's like 15 minutes or 30 minutes, and I totally appreciate why that's done, particularly if it's a terminal sample. But ACTH actually has a much, much faster time dynamic than steroids. It's up and down, so in terms of like restraint, it responds in under two minutes, and in our hands it's down in like 15 minutes. It's fast. But what it does stimulate, of course, is a longer, slower and sustained rise in corticosterone.

So in the experiments we see, particularly with cannulated animals, the opportunity to take samples that are sensitive around the peaks and falls of where you're going to find ACTH separate from corticosterone are just wide open and can be taken to advantage.

And then I'll take this point to say that the urine collection and measurement of corticosterone, to my mind, is a fabulous tool. And what it brings to mind also is to

recall that corticosterone is carried by a binding protein. When you measure corticosterone you're actually, in most cases, measuring total. But the bioactive portion, of course, is free. So you have to reinterpret this, particularly in a chronic state where the binding protein will decrease.

And the urine samples are fabulous, again, because they give you an integrated measure, but you're measuring free corticosterone, the bioactive fraction being seen. So there's a lot of rich possibilities in the kinds of data that are coming out, but they could be tweaked, be a bit more sensitive.

DR. PORTIER: Dr. Krishnan, and then Dr. Hayton.

DR. KRISHNAN: I just wanted to -- to O'Bryne's comment in relation to those two slides yesterday. You saw some of the magnitude of difference between the NOAEL water concentration sources and the monitored

concentrations. I think that's what you were referring to at some point.

Just look at the last one, or the one to the right-most comparison, which was the chronic one. There was a factor of about 6,000-something, where the NOAEL is all the way up there, NOAEL water concentration, monitored community water concentration was here, and the factor was about 6,000-something, 6,200 or 500, whatever.

The classification I was trying to get when you all walked out on me at the end for the session there --

(Laughter.)

DR. KRISHNAN -- was that the expected factor, or the target factor is 5,000. That's what wasn't on the figure. The difference was being shown as 6,000-something. But the expected or the target factor in the risk assessment was about 5,000. That was my comment yesterday.

Because of the uncertainty factor

on the source contribution that's accounted for in the NOAEL to bring it down to a human relevant concentration. So that would make a difference. Because down below is what actually the humans are consuming as water concentration. Up there is you take the dose given to the animal and then try to solubilize them in the water that can be consumed.

So, you know, that -- I just wanted to clarify that. So I'm trying to --

DR. LOWIT: Kannan? Dr. Krishnan?

DR. KRISHNAN: Yes.

DR. LOWIT: We had some questions about Dr. Pastoor's slides also, about the differences in some of the numbers. But the -- but one thing that I know to be true is that the -- whether -- the magnitude of the totality of the uncertainty factors is not 5,000, and that's the value that I think that you're quoting. It's either 300 or 1,000 --

DR. KRISHNAN: Okay. Well, the --

DR. LOWIT -- depending on the --

DR. KRISHNAN -- 5,000 I quoted is based on what he presented. He said 1,000 was the factor involved in that particular assessment, including a 10 for children. And then he said 20 percent was included as a source contribution. That was presented during his talk, and then there was some other comments --

DR. LOWIT: Yes, and --

DR. KRISHNAN -- that came after.

DR. LOWIT -- too bad Dr. Pastoor couldn't clarify those numbers with the microphone on, because I think some of the numbers he was quoting, I think they were getting mixed up, and there was some differences in between the OW and the Pesticides Office procedures that got blended in that conversation. It's really confusing.

DR. KRISHNAN: Yes. No, so I just wanted to, you know, keep in that mind. I didn't want that to be one of the driving forces of our discussion, or how you view

things.

The other thing is that the focus on the toxicity pathway is based on the National Academy of Sciences recommendations, and is unquestionably on a solid footing the way the Agency has put it together. But one of the things that worries me a bit is that I hope there -- the assessments won't take us down a pathway of establishing no observable perturbation effect level without, you know, regard to the no observable address effect level.

So I think at some point as this matures along, that relationship has to be strengthened and clearly presented, such as a like an AUC of an LH surge works in an organism level effect or something, so that the perturbation levels for specific pathways that are chosen, even though, you know, some are blurred along the line with respect to the outcome in the animals.

As long as those are chosen for a

benchmark, those analyses or other kind, with particular reference to, or in relationship to the organism that will affect. I think that will be reasonable. I just wanted to add that comment.

DR. PORTIER: Dr. Hayton?

DR. HAYTON: I have a question for the discussion, and I'm trying to understand. I know we can measure perturbations in the HPA/HPG axis signals, at what point do you think those constitute an adverse health effect? I mean me speaking into this microphone, my corticosterone probably is going up, but is --

(Laughter.)

DR. HAYTON -- is that an adverse health effect? And it seems to me there's not much linkage between all those signals and adverse health. And I ask this because it's important for a later question, so maybe it gets addressed in later questions too, but any perspective on that?

DR. PORTIER: Dr. O'Byrne?

DR. O'BYRNE: I think acute effects are not important. I think there's ample evidence of that. But chronic elevated levels are putting you at risk, and there's huge amounts of clinical data for that in both men and women in terms of infertility and the inverse relationship.

However, whatever method you use to reduce those stress levels, whether it's cognitive behavioral therapy, talking to women and just giving them a nice environment to relieve their tensions, et cetera within -- for example, within fertility clinics, you can restore fertility quite easily. I mean, again, there's some magnificent data coming out of the US from Sarah Berger's lab where you can get an 80 percent restoration of normal fertility through a short course of cognitive behavioral therapy. I mean it's just mind-blowingly astonishing data. So chronic levels, yes, are a problem.

DR. PORTIER: Dr. Regal had her hand up, and then Dr. Selvage, and then Dr. Williams.

DR. REGAL: Just a quick comment in terms of Dr. Pruett's data where he showed that there were alterations in immune system function that correlate -- or immune system parameters that correlated with area under the corticosterone curve. So that under his situations where he was dosing the animals, there was some function change.

DR. SELVAGE: Dr. Selvage. I concur with that. I just wanted to add that, you know, it definitely is the chronic activation of the HPA axis that you want to worry about, and there's just a huge laundry list of problems associated with that, including immune system dysfunction, also reproductive dysfunction, psychological disorders, et cetera. So I think that is what we should be mostly concerned about, if we're going to be concerned.

DR. PORTIER: I think Dr. Lowit wanted to slip in before Dr. Williams.

DR. LOWIT: Yes, we have -- Ralph and Dr. Cooper and I were just, I think at the same time, having the same question, and it may not go here. As I've scanned the questions, it may actually go in 1.9, but I'm going to ask it anyway because it's relevant to the discussion.

The discussants are talking about acute and chronic. Where do you turn the -- where does -- you turn from acute to chronic, and keeping in mind what our needs are here, to define some temporal window to establish regulatory limits. So what do we mean as chronic, and when we go from a whole bunch of acutes to something that becomes chronic?

DR. PORTIER: So we'll keep that question in mind, as Dr. Williams come in.

DR. WILLIAMS: I just would like to ask for clarification on the discussion with Dr. Krishnan and Dr. Lowit. I didn't

understand what you were getting at terms of the 5,000 and 1,000, and what do you really mean, like for the more lay person in that? Do you mean that the doses used are even higher than they ought to be, or should the relevant doses be lower, or what --

DR. LOWIT: Well, if you remember from Dr. Pastoor's series of slides, what he was doing in those slides was to compare the atrazine in drinking water monitor levels that Syngenta has calculated with their box and whisker plots across different durations. So basically what Dr. Pastoor was trying to do in a couple of slides was to put everything that we're talking about in a risk assessment context, in bullet form that was quick.

And it's -- there's enough nuance in the differences there that there's life stage, there's time, there's different offices. He had things on there that come from the Pesticides Office and from the Water Office, and so it was a blend of a lot of

different things on one slide, and he just didn't have time to really go through and explain what all of the columns were. And so in the haste, it just got really quick, because everyone was tired.

So what he was doing in that series of slides, and I don't want to speak for him, but I think this is what he was doing, was to compare different aspects of what goes into a risk assessment to the water monitoring data as a reality check, as a reality check to see how the doses in the animal studies compare to what humans get in the water, and actually US water monitoring, and to -- I believe his point was to show that the magnitude between what's in the animal studies that's used as that line in the sand versus what's in the monitoring data are pretty large differences in there.

But what got lost in the speed was the nuance of how the uncertainty factors are used, how the conversation from a milligram to

a kilogram to a ppm is done across different life stages. So I think that's where a lot of the confusion has been of how some of those numbers were derived.

DR. WILLIAMS: So if you take sort of the number of the animal exposure that's closest to some adverse affect in any life stage in humans, is there anything that's closer than something like 100,000 that he was talking about? Like at what point are we -- like how far off are the animal studies --

DR. LOWIT: Oh, it's much closer --

DR. WILLIAMS -- from any possible risk to humans?

DR. MENDEZ: It's closer to 100,000 --

DR. LOWIT: Nelson Thurman's going to come up here.

DR. MENDEZ: If you could just give us a second. But I just wanted to add to what Dr. Lowit was saying in terms of the

uncertainty factors. When the Office of Pesticide Programs calculated drinking water level of comparison, we consider the body weight of the individual drinking, for adults, two liters of water. The Office of Water does it slightly different with a 20 percent contribution coming from the water. So that's where the difference between the health advisory levels and the DWLOCs come in.

DR. LOWIT: And it's further complicated by the MCL comes from a fairly old dog study whereas all the Pesticides Office work comes from the mode of action that you've heard quite a bit about. Not the HPA part but the HPG part.

DR. PORTIER: Instead of kind of going off on this, Carmen, maybe at the break we'll sit down and let you explain how they kind of use uncertainty factors and clarify it. But I think most of the panel probably understands this? No? Okay. I just wanted to double check.

(Laughter.)

DR. PORTIER: So we'll take two of you because -- no, I know Ken Delclos knows this. Okay. Well, then I stand corrected. Continue.

DR. LOWIT: It might actually make more sense if we want to either have Dr. Pastoor explain it himself, or alternatively a group of us from the Agency can, at the break, throw a slide together of what some of the pieces are --

DR. PORTIER: Why don't we do that.

DR. LOWIT -- if that would help?

DR. PORTIER: Dr. Horton?

DR. HORTON: No, all we're really trying to get is a sense -- we don't need a detail, we're just trying to get a sense of how close we are to safe versus unsafe as we go -- as we look at those box and whiskers relative to what --

DR. LOWIT: That's not a two-word

answer. It's --

DR. HORTON: Okay. Okay.

(Laughter.)

DR. PORTIER: I'm going to ask Dr. Greenwood to step here for a minute, because he's usually pretty good at explaining stuff.

DR. GREENWOOD: I don't want to try and explain, I think we need to get this -- I think it is important because we've already heard about, from several people, about the importance of pharmacokinetics, and that's something the three of us here have been discussing on and off looking at the papers.

It's not straightforward to interpret them, and I think it is important that everybody understands the relevance of the levels in the water compared with the probable or possible adding under the curve in the plasma. We want to try and relate what levels of dose given to the animals gives you those areas and what sort of concentrations in

the water are giving what sort of exposure in the gut to humans. So in order to sort this out, I think we all need to be clear about what's going on here.

DR. PORTIER: My question is, is this the question to be doing it on?

DR. LOWIT: That's for, I think, for you to judge as the Chair. But if it's going to cause at least certain numbers of the panel to struggle through the responses to some of the questions, I think at the break we -- that, you know, that the Agency takes, you know, an extra five minutes and we throw something together to help you understand a little bit more of the risk assessment process. But otherwise, I think the better place in 1.9, but that may be tomorrow.

DR. PORTIER: No, I think we should do it today, but I'm just trying to figure out whether in this question, which has, you know, six very specific questions, we're king of getting side-tracked, and I

don't want to get too side-tracked on that.

Dr. Krishnan.

DR. KRISHNAN: I think one of the reasons for the side-track being whether the doses we are talking about are really out of whack or is there any relevance at all, because with some of the thousands of factors we saw, I think that's where we got a bit confused.

If I can take you all -- I'll only take two seconds -- page number 5 of Pastoor's presentation, if we can get back to that, that's where the factor is. Well, if you can find that, and we can --

DR. PORTIER: That's what I was thinking, if EPA could bring that up after the break, we'll have a 10 minute presentation by EPA so they can clarify that. How about that?

DR. LOWIT: Yes, we may have to make our own version of that one, because we weren't sure about some of what was -- it was just a confusing presentation. We just have

our own version, if that's okay?

DR. PORTIER: That's fine.

DR. LOWIT: I hope Nelson's going help.

DR. PORTIER: Okay. So we'll come back to that and, you know, we'll clarify that definitely today, before lunch.

Okay. So if we can get back to these questions. Where are we? Dr. Akana.

DR. AKANA: So, when you talk about what are the health concerns of acute and chronic for a single administration of atrazine that's not pharmacological, in the adult I don't think there are major health consequences to a single exposure. In the adult. And then later in the session we'll be talking about doses given to prenatal or gestational or pubertal animals, and that's a whole different ball of wax.

In terms of the chronic measures, the problem with looking at what are the health outcomes on chronic exposure is you

have a wealth of endpoints to look at, all with different time domains. So you could pick one, any one. You could pick reproductive, immune, body weight, which is one of my favorite cheap endpoints, and what I would imagine is, will you hypothetically want that water exposure chart that we've talked about, and floating above it you want outcomes.

What is the first -- what are the responses of the animals to the LOAEL? You know, how major are we talking about, a thousand-fold response in the animal, you know, what's our concern here. So while we construct the water one, what we've one day want to work towards is a hypothetical outcome box and whisker diagram floating right above the water.

DR. PORTIER: Dr. Selvage.

DR. SELVAGE: Yes, I just wanted to make a couple of comments regarding this issue, one being atrazine -- personally I'm

not so concerned about atrazine levels in the plasma, per se. You know, this is stuff that's going to get into tissues, you know, I'll get -- it gets into the brain, et cetera. And if I remember correctly, it hangs out in the brain for some time. I don't think it gets metabolized in the brain very quickly, so I think we need to be concerned about levels in the brain, et cetera. I think that's important when you're talking about this issue.

The other comment I wanted to make regarding chronic versus just acute HPA activation is we almost have to have two discussions, one concerning males and one concerning females because of the lack -- supposed lack of habituation you see in females.

DR. PORTIER: Any additional comments on this? This is really good. The problem with the first question is we always want to put everything under the first

question, and I'm trying to avoid that. I think -- you know, I've heard a lot of good comments from this.

Dr. O'Byrne, did you get good notes on this? You think you're going to be able to write up this first one?

DR. O'BYRNE: I'll borrow yours.

DR. PORTIER: I don't have -- I have three of these -- oh, right yes. Yes, I was going through your six questions; I think they've answered -- we have answers to your six components of this first question. It seems that there is some agreement, concurrence with the hypothesis, but there's things around it that the panel wants to fill in with your model. I guess that would be a fair thing to say.

Anna?

DR. LOWIT: I think we'll sort of hold back any other clarification questions till we hear the next couple of questions because I think combined we may have a

clarification or two.

DR. PORTIER: Okay. With that I think we'll move on to Question 1.3, which is another multi-part question.

DR. LOWIT: And, Joe, if you could come do your --

DR. PORTIER: Magic.

DR. LOWIT: But I'll read from the paper copy, I guess.

DR. PORTIER: By the way, I take responsibility for asking the Agency to read the full questions before the panel because the questions are not one question, they're multi-part questions with conditions said at the beginning, and I wanted to make sure we all saw those and were aware of it as we began our discussion. Sometimes we simplify the questions, but this was not a good case for simplifying the questions.

DR. LOWIT: The Agency has preliminarily concluded that atrazine directly targets cells within the HPA axis, Section 3.2

and 3.3.2.3 of the draft issue paper -- I don't know if I got all that or not -- and the increased activity of the adrenal axis does not reflect non-specific stress. Evidence for this conclusion is based on the following observations:

Bullet number 1, several studies report increased adrenocorticotropic hormone, ACTH, and corticosterone levels immediately following a single explore to atrazine and its two metabolites -- I won't say the whole names -- DIA and DEA, but not DACT. This data comes from the Laws, et al., Fraites, et al., and Pruett, et al., studies.

A single intravenous administration of DEA in vivo also causes an immediate increase in ACTH and adrenal hormone release indicating that a chlorotriazine-induced GI streass is not driving the hormonal response. The adrenal corticosterone response to continued oral exposure to atrazine did not habituate in rats or mice. And that comes

from the Fraites, Laws, and Pruett papers.

Also, although atrazine induced a rapid increase in ACTH, prolactin secretes was not affected in the same animals. This is unusual in that an increase in both ACTH and prolactin are typically observed in response to a number of physiological and psychological stressors.

Please comment on the extent to which the available evidence supports the preliminary conclusion that atrazine and its intermediate metabolites, DIA and DEA, induce changes in the HPA axis as a result of a direct action on the HPA tissue, and such tissue -- such changes are not due to a generalized or non-specific stress response. Are there data on other substances that would support this conclusion?

DR. PORTIER: Dr. Selvage?

DR. SELVAGE: Okay. That's really almost a one-part question, when you think about that way.

We can be optimistic. Let's see, first I wanted to just give a preliminary couple of remarks. I'm going to -- for my answer I stuck to a mechanism of action. I'm not really considering dose so much, because the doses are obviously very, very high for this.

Another thing I wanted to say, and this is just my own personal view, is the term non-specific stressor, generalized stressor. You know, there's certainly stressors that activate the HPA axis a number of ways, but I think for each stressor, I think that there are fairly specific, for instance, neuro pathways, that can be activated, and can be delineated with lots and lots and lots of work. So I think that I'm just not a big fan of the non-specific stressor. However, I do realize that gets used a lot, so I just wanted to say that.

Okay. And so then to get on to the question about whether there's a direct

action on HPA axis tissues, once again, with the HPA axis, in response to a wide variety of homeostatic challenges, you know, just about any homeostatic challenge you can throw, an animal you can get an HPA axis response. So to determine a specific action, per se, is not easy, especially a specific action on HPA tissues, because there could be multiple actions on specific HPA tissues than actions on other tissues that in turn affect the HPA axis.

So I guess the short answer to this is, you know, I don't think there's enough data yet. We need more information regarding atrazine's affects at a variety of levels. So, and also interpretation of the results presented so far is a little bit difficult for me because we've got -- we're using different strains of animals and different species.

So we've got evidence in mice and we've got evidence in, well, now, with Dr.

Handa's work, three different -- at least three different strains of rats, and different strains of rats all will response differently to the same stressor and, you know, even the vendor you get your rats from can affect how they're going to respond to a stressor.

So it's difficult to kind of make a -- you know, take all this information and think that it all really, you know, fits together in a cohesive manner with all these different animals. On the other hand, you've got a bunch of different animals and your getting a similar response. So that's also a positive.

So I guess -- let me see, once again, you know, I guess it's possible with the information available that the HPA disruption by atrazine could be due to a direct affect on HPA tissues. The evidence that's been giving the supporting evidence, first of all, is simply that atrazine activates the HPA axis when given

peripherally, you know, and that's obviously an important finding.

Also, other stressors such as restrain stress, were able to cause prolactin release, whereas atrazine administration did not cause prolactin release, and that's given as supporting evidence.

Also the fact that sub-diaphragmatic vagotomy didn't block the HPA axis response to atrazine at least rules out gut irritation as the means of activating the HPA axis, and then we have the information that when we give, I believe it was DIA was given IV, you're still able to activate the HPA axis. Some other information I would say would, you know, at least preliminary support, looking into this hypothesis, is that atrazine can get into the brain and can have affects at the brain.

Having said that, I just think there's not enough evidence to really say that atrazine is acting directly at HPA tissues,

although you can't say it is either. And with -- for instance you've got this habituation of the cort response that did not seem to occur, but you got -- there's habituation of the ACTH response, which to me makes me wonder is something going on at the level of the adrenal gland, and there's some information, I believe in amphibians and fish, that actually -- and I believe this was discounted by the EPA, probably for good reason, but those two studies showed actually a decrease in cort synthesis in those species.

So if that's actually true, the you probably are working at the hypothalamic level to activate the HPA axis, or, you know, or some other level and not directly at the adrenal gland. However, more information is needed.

So, you know, I think there's a lot of alternative hypotheses that need to be investigated. The first one, which I've already brought up, is I think you might have

an overall increase in HPA responsiveness that's been to stressors in general in these chronic HPA -- in these chronic atrazine administration studies, which, once again, brings us back to why it's important to get baseline cort measurements and other time points for cort.

Also, in the studies where we're looking at chronic atrazine administration over four days, or Pruett's study over 28 days, there were no measurements made in between. And you can get alterations, you know, in the HPA axis over time that we just might not have seen.

And I think you can't really do this so easily in mice, but in rats you can certainly put in an IV catheter and keep it patent for a decent amount of time, and you could be making these measurements. So I think that's something that could, and should, be done. So, you know, you put in an IV catheter and you indicate many draw blood

draws. I mean they're not going to stay patent forever, but you can still do that and it'd be helpful.

So there's also, you know, just many other parameters that I think atrazine could be affecting that could activate the HPA axis. You know, you could get physiological changes in the periphery that I don't think have been examined. I did see one paper that indicated atrazine actually caused hypotension, a decrease in blood pressure. A decrease in blood pressure activates the HPA axis. I think that would need to be examined.

Let's see, also I think sympathetic activation, activation of both central catacombing noradrenergic system needs to be examined more thoroughly, and also we need to look at, in these animals, and you could do this if you have an IV catheter in place, at least -- it's a little tough, but you want to look at norepinephrine and norepinephrine secretion in the periphery,

because that can cause a lot of the affects that have recorded, especially the immune system affects.

What else do I have here. Once again, I think it's important to look at more central mechanisms, as Dr. Akana said, CRF, vasopression, once again norephinephrine. Also I think looking at the affects for PDE, phosphodiesterase inhibitor, inhibition could be looked at more thoroughly in the pituitary, as well as in the brain because, you know, a cyclic AMP, when you're increasing cyclic AMP, you know, if you're doing this fairly non-specifically, you could just be, you know, causing a myriad of effects we haven't seen.

So those are my main comments regarding the specific action there. You know, I think other people will add a lot more to this. In terms of evidence for other substances, simazine was tested, which is like some of the atrazine, and that had lesser, I believe, activation the HPA axis, but it did

activate the HPA axis acutely. Was that done chronically? I don't -- simazine? It was just -- okay, it was acute. Okay. But it did have an affect, you know.

Also, there's other, you know, molecules like atrazine that can get into all sorts of different tissues, you know. It's obviously a different molecule and probably will get in more tissues. But ethanol, for instance, the number of way that agents like that can activate the HPA axis are legion. So I just think it's way too early to draw a conclusion that you're directly affecting HPA tissues. So give Dr. Cooper a lot more money to do --

DR. PORTIER: Dr. Akana.

DR. AKANA: First off, in the HPA world, if you're interested in an agent that stimulates ACTH and you want to know if it's non-specific, the answer is there's, in my world, no such thing as non-specific stress. You have uncontrolled stress, you have

parameters coming that you don't know about. So in the HPA world, half the experiment is controlling your situation to exquisite, immaculate basal levels.

And so in both acute injection and in chronic, it is really important to know you have your animal in a basal condition. And in many cases, that's corticosterone. Now I know that's an inhibition against sampling from animals if you don't have a catheter in, but if you look at the literature, if you really, really need to know, you can measure it in urine, you can measure it in fecal boli, if you really have to, which you can.

So I think what I can say is that in response to acute injection, does atrazine stimulate ACTH, I go for that. Yes, it definitely does. And the more recent studies, to me, are much more credible because they show a wonderful control of basal ACTH and corticosterone as an initial condition. And that's critical in my book.

Let's see, on the question of gastric distress, I don't think it's quite definitive yet that the response to atrazine is not in the gut; we have one nice vagotomy experiment. But in the feeding world there's an exquisitely simple experiment, is because rats, and I believe mice, cannot vomit. If they are given something to eat or gavage, they can't get rid of it. So what they can do is they run and will eat inert clay, kaolin. And you can measure this. So you provide in the cage a source of kaolin, and you measure how much they eat. And it's very old-fashioned and very simple.

And in the feeding world they have many, many, many measures for measuring the gastric distress, and a lot of them are behavioral. And I think the atrazine studies may be ready to look at different kinds of behaviors as very sensitive indicators of what kind of actions they're getting.

I looked in my time, I didn't see,

what are any affects of -- on behavior with atrazine administration?

DR. COOPER: I'd just add one quick point. Michell Hotchkiss, working in Dr. Law's lab, has done the pica studies, and -- right. So we have that data, it just hasn't been submitted yet.

DR. AKANA: Oh, that will be really useful.

DR. COOPER: And I guess without giving the specifics in general, it turns out that you see no change in pica behavior, no increase in inert --

DR. AKANA: You see no change. That's promising. Thank you.

That also reminds me that in very old traditional studies with chronic stress, at the end of the experiment they would examine the gut for ulcers, you know, very classic, very old, and very direct in this particular kind of study with gavage.

Now when we come to the question

of habituation, this is a very interesting nugget in this study, and it raises some possibilities. First of all, in the male/female difference, we'll remember that females have a lot more of the binding protein CBG. So this may be obscuring some of the responses we see. I would love to know what is the actual free corticosterone response in those females.

And then immediately the second thing is, I'm intensely interested in those adrenals, and I think it's ready for studies of adrenal sensitivity, both in vivo and in vitro to be initiated. And that might help clarify what's the male/female difference.

And then in my particular world, when we talk about habituation and the HPA system, there is one particular brain nuclei, the peri-ventricular thalamus -- excuse me -- that's correct, the peri-ventricular thalamus of the hypothalamus and I wonder if there's some special susceptibility in the female.

And then on the concern about prolactin, from -- I'm to a prolactin expert, but from my review of the literature, the prolactin response is not always obligate with a stress response. And also, depending on whether it's a predicable or non-predictable stress, that's GeneQuant.

And also the time course can vary from that which you find with corticosterone. So depending on when you sample, you may miss a prolactin response. There's also a diurnal difference in the timing of prolactin in response to, say, restrained stress. So the prolactin divergence, per se, do not concern me at this time. And, oh, the last point which may be of interest is that the prolactin response can be very dependent on the dopamine total for the animal. So that's something to just flag in the back of your mind.

DR. PORTIER: Dr. O'Byrne -- LeBlanc?

DR. O'BYRNE: I also agree, this

business about non-specific stress really does muddy the water and should be discontinued. Adding uncontrollable stress muddies it just as much, in my mind. I mean people who work in the stress world are plagued by sub-clinical stresses, which have a nasty habit of summating and synergizing and screwing up your experiments. So stress is a stress, and it depends on your ability to detect it.

In terms of the dynamics of ACTH and cort release in response to atrazine seem pretty normal to me. The business about the ACTH not being maintained and cort being maintained doesn't necessarily surprise me, but from -- the HPA axis is not my baby. But I do recall being confused by the literature, graphs showing that ACTH doesn't always increase.

So I think there are changes in sensitivity to the -- of the adrenal level, and I think this is an amazingly dynamic system. So you don't necessarily always need

the brain to get the same degree, I think, of cort response. It's a very sensible system. We can switch over to a semi-automatic, peripheral system, I think, in the context of chronic, ongoing, or repeated stresses.

In term of the tools that could be applied, we've got CRF1 and CRF2, not counting mice. They should be used in these sorts of experiments so you can get -- you know, differentiate the involvement of the receptor sub-types. And even if you don't want to use, not counting mice -- and I have problems with them, they're not as clean as people think they are -- there are amazingly selective CRF1 and CRF2 receptor antagonists, which are extremely selective, extremely potent, and those are tools that should be used.

In the context of prolactin, I don't know anything about prolactin, to be honest. But it does seem a little puzzling to me. But trying to do some work last night, I discovered an amazing paper in 1990 -- I can't

remember where it was published -- Neuropeptides, which is, you know, a reasonable journal.

But CGRP, given intraperitoneally and subcutaneously, blocks the prolactin-induced -- the stress -- that blocks the progesterone increase in response to stress -- I can't remember what the stress was -- thermal stress and injection stress. And that's quite interesting because I did a bit of work on CGRP, which is quite potent in terms of stimulating the HPA axis and inhibiting the HPG axis. That's what we discovered. This is calcitonin gene-related peptide.

I mean the work didn't go any further, we just beautifully described it, and it fitted within the stress arena. But it was quite interesting to discover this paper, that it blocks the prolactin response.

And I think Susan's comments about the dopamine system in the brain controlling

prolactin, there is a paper from Garla in 1990 -- unfortunately it's in Life Science, which is a pretty awful journal, so I'm not quite sure whether it'd be worth quoting -- but there they talk about the increases and decreases in prolactin in response to different stresses. So I think there's very old literature on this discordance, which perhaps shouldn't be of great surprise, that's what I read.

DR. COOPER: One of the things that we find consistently within the hypothalamus, especially when we looked in the mediobasal region when we did our catecholamine work, and it's published data, is that we do see the one thing that hangs in there as the dose goes down is an increase in dopamine, and these are, again, acute exposures.

This is in contrast to the neurotox papers that you saw that implied there was dopamimetic neurotoxicity. We see

within the mediobasal hypothalamus an increase in dopamine concentration in DOPAC, both of which, of course, you know will inhibit prolactin. And in our 2000 paper you saw post-dependent decrease in serum prolactin in estrogren-primed animals. And the other thing about that is that seemed to be a brain effect because of certain dynamics between how much dopamine -- I'm sorry, how much prolactin was present in the pituitary of those animals. So what you're saying is consistent with that.

DR. PORTIER: Dr. LeBlanc.

DR. LeBLANC: I agree that there's an accumulating body of evidence that would indicate indeed that atrazine is affecting the HPA axis in a manner that's independent of a generalized stress response. But I think the key word in the statement we have on the board there is preliminary, preliminary conclusion and I hope that's emphasized by EPA. And I think we've heard that from everyone has spoken, the evidence is accumulating, but as

there are only -- I don't think we can conclude at this point that that is indeed the case. We need more information.

And I think it's important to recognize that in terms of a generalized stress response, it's not driven only by ACTH, and the EPA had addressed that and they've looked at the prolactin response and they use that as part of their evidence, and I think that's good. But there are certainly other hormones that should be considered.

And one has, thyroxine, we've heard at least two studies with which thyroxine levels have been evaluated, and typically thyroxine levels are increased with a generalized stress response. However, the studies that we were exposed to, thyroxine was either not affected or decreased. So, again, that would argue against a generalized stress response.

Another hormone is growth hormone. It's typically decreased in stress response,

and I don't think anyone's looked at growth hormone, but it might be something to consider as additional evidence as to whether or not we're looking at a generalized stress response. And I think another interesting aspect with respect to growth hormone, in the rat at least, is that it -- growth hormone regulates sex-specific cytochrome P450s in the liver of the rat that could impact corticosterone metabolism.

I'm not sure that they would affect atrazine metabolism, but could affect corticosterone metabolism. So it may be a very -- it may provide some additional linkage, or explanation that relates to the sexual -- sex differences that we see in some of the atrazine responses like habituation.

Everything else I have here I think has been covered. I'm not going to be repetitive. One other point, I think it's important also to recognize that there could be complexity here that we're not recognizing,

and maybe I can best explain it with an example. Perhaps atrazine is indeed eliciting a generalized stress response, but over and above that it's impacting TSH levels, which are impacting thyroxine levels.

And so we're saying, oh, look at this profile, we're not seeing a generalized stress response. But maybe we are, but in addition we're seeing something else. As Dr. Bucher said earlier, toxicity pathways are rarely linear, typically they're webs, there are many things going on. So there could be additional layers here that we're really not considering; we're sort of taking the easiest path in trying to explain it in that manner. But I think we need to recognize that there could be multiple things happening. And I think I'll stop there.

DR. PORTIER: Additional comments from the panel? Dr. Selvage?

DR. SELVAGE: Yes, I just wanted to add a couple of things. First of all, I

realize it's extremely easy to sit here and conjecture and, you know, dream up experiments, and it's much more difficult to actually do them. So I want to take -- I want to make sure that gets said because we could probably sit here all day and come up with 10 years of work. But --

DR. PORTIER: If you didn't do that, you would be a unique panel.

DR. SELVAGE: The other thing that -- I just wanted to add one more thing that might be worth looking at, but the problem with having different strains of animals confuses the matter a little, is that it looks like with this smaller -- obviously in different strains of animals, but with the smaller dose of atrazine, you got, I think it was 75 mgs per kg, you got at least double the ACTH response in females versus 100 mgs per kg in males, so.

And you're measuring the measurements in the chronic studies, and in

the acute studies, the females were done on proestrus at a time when, relatively speaking, estradiol levels are going to be higher. So they might -- that's thought to be -- estradiol is thought to increase HPA activity, so I think it might be worth looking at central sex differences as well as adrenal sex differences in response -- HPA responses to atrazine.

DR. PORTIER: Any additional questions? Dr. Akana?

DR. AKANA: I agree that looking at a stress response to atrazine is really hard because you're looking at a web, an ensemble of responses. So a general philosophy is to look at the temporal pattern, see which one changes first. And then a second general strategy, which many labs do, is you take down one system at a time. And then if you're really clear -- for instance adrenalectomy and corticosterone replacement. And then if you get really bold, you do two at

the same time. So there are some approaches that might be yielding.

DR. PORTIER: Anna, you had -- Dr. Lowit?

DR. LOWIT: Yes, I was just going to take the opening from Dr. Selvage to -- it's fascinating to sit here and listen. I'm learning a lot about the stress response and the HPA that I don't know if I ever really wanted to know or not, but I'll soak it all in.

Just to I guess push back a little bit to -- as you're thinking about the comments and writing the report, about what our -- what the purpose of this is, and about the value, the added value of understanding every tiny detail of a mechanism when, at the end of the day, the Agency needs to come up with a scientifically supportable risk assessment.

And what that means is that we're going to need to derive a series of values

that are protective of human health. And the value of -- the added value of understanding every component of that web that we keep hearing about in that need. So I just ask you to put that in the back of your mind as you make these comments.

DR. PORTIER: Dr. Selvage?

DR. SELVAGE: Yes, that's why I prefaced this by saying I wasn't taking dose into account, so.

DR. PORTIER: Dr. Reed.

DR. REED: Well, I was going to say this to Question 1.9, but I think this is a good place. I'm not going into 1.9, but I think this a good place to just, you know, bring out some of the endpoints. So at the very tail end of this hypothesis, the endpoints that we're seeing in animal studies, in dogs, there's cardiopathy, there atrial fibrillation in rats, and these are sort of the endpoints that comes out as, quote/unquote, more or less more sensitive

than other endpoints. Rats, extra-medullary hematopoiesis in the spleen, renal effects, neuropathy, just throw out some of the endpoints and see if you can connect the dots at the very end.

DR. PORTIER: Any additional comments? Dr. Lowit.

DR. LOWIT: We don't want to get into Question 1.9 here. I didn't mean to do that with my comment, it's just the opening was there and I felt like it needed to be said. But our desire with -- the last atrazine risk assessment really set a strong standard by using an LH endpoint. As a precursor event, we are far removed from any of these more serious outcomes, and we plan to follow that same approach. The ultimate question is, what does that place in the precursor events, to be a precursor to all these other outcomes that may come up.

DR. PORTIER: I'm not seeing a lot of additional questions, so I think we'll ask

EPA if they got out of this what they were looking for.

I mean, you know, my take on this is that they don't like this non-specific stress term and they really kind of have explored -- like you said, they've explored the stress question quite a bit trying to put your experiments in a --

DR. COOPER: If I could just make one comment, final comment. Yes, I think these have been extremely helpful. I think that some of the directions that have been suggested this morning are already being pursued. We're one lab, three PIs, and we published the first paper on the adrenal access in November or December, I forget exactly when it came out. So this is relatively new to us. We're not experts in HPA by any means.

The connection was there in the literature. We continue to be confused by the acute versus chronic outcomes, and the onus is

on us, and I think others, to looking into the connections, if they are there. We can't ignore them. They're big flags that at least in the endocrine world are important to us. So all this has been helpful.

The suggestions about knock out specific receptor blockers for CRF, measurements of CRF, AVP, these things are hitched into. And I have one technician, and these ladies behind me have one each too, so if we had the world enough and time enough, we may have been able to address those things. But this is useful.

And we see this as not our research, per se, it's a research area that there's a lot of very well-qualified labs around. It's an extremely interesting series of events that occur, and the more we can enlist the basic scientists in these kinds of questions, the happier we are.

One other thing about dose, I think we constantly are dealing with the

issues of dose and route of administration of those kinds of things. They're not foreign to us, but sometimes the restraints of the question put us -- so we're looking at little parts of the bigger picture and you see these, and of course those are all very good comments that we've received about that, and we appreciate them.

DR. PORTIER: Let's take a break until 10:30.

(Whereupon, the above-entitled mater went off the record at 10:14 a.m. and resumed at 10:33 a.m.)

DR. PORTIER: So, Dr. Lowit, I was trying to summarize Question 1.3 and kind of looking over my notes, and I get the feeling that Dr. LeBlanc's comments were probably the most pertinent ones in saying there's an accumulating body of evidence, but it's preliminary and there's need for more information.

And we got a lot of suggestions of

where the additional data, addition information will come from. And I've talked to the panel members, and the -- kind of the details on that additional information that they'd like to see and the stress-related impacts, that that's all going to be put in the report.

And I do think there's a general acknowledgment of what EPA's trying to accomplish with this in terms of their need for a risk assessment. And you're going to see some of that. It may extend then to academic research needs, but there's -- I think there's a good recognition of where you need to go with this.

DR. LOWIT: I think some of these issue will -- what I'll call the collision between the risk assessment world and the academic world will probably reappear in Question 1.9, so I think I'll hold back sort of pushing any more around that, because the question is when is enough enough.

DR. PORTIER: And I'm making kind of a list of issues as they come up here that'll come back up again in 1.9 where we starting looking at issues like acute versus chronic definition, how do we look at that, and health effects and how do we define those health effects. And so we'll just kind of keep track of those and that'll be our catch-all question.

So with that we're going to move on to Question 1.4.

DR. LOWIT: We threw together a quick slide to sort of do the -- but we have to either switch the video thing, or get it to John's computer. It's on my machine.

DR. PORTIER: That's okay. It'll just a second.

(Pause.)

DR. PORTIER: Now we took a break and everybody destressed and got their cort levels low, now don't -- you know, don't raise it just yet.

DR. LOWIT: Except Nelson and I, because we've been sitting here doing this.

Okay. So this is not what Dr. Pastoor showed, you know, thinking where are those little box and whisker plots and where are the margins of difference. Intentionally not there. We, as a team, as an agency across three offices, came into this meeting focused entirely on science, and focused entirely on a lot of very difficult science that may or may not have a lot of impact on what is already a very complicated risk assessment.

And so you may, after seeing Dr. Pastoor slide, notice there's a vast crevasse where our documents to you don't talk about this stuff, and that's, in many ways, intentionally. We want this meeting to be about science and to be about the science that informs our decisions, not necessarily about the decisions that we haven't made and haven't figured out how to make yet. That's why you're here to help us, guide -- to guide us

to make the right ones.

So you haven't heard us talk about points of departure, except for I think two bullets in Liz's slide yesterday. We haven't talked about uncertainty factors, we haven't talked about margins of exposure, we haven't talked about RfDs, we haven't talked about any of that because we want this to be about science and it's very complicated all by itself.

And when you bring in a lot of very complicated policy on top of the science, it gets confused. And so we want this meeting to be about science and about the science that will help us to go in different directions to inform the decisions that we have to make.

So what we're getting back to is not what you -- I don't think what you've asked for, but I think it's what you really need.

And that may be a little patronizing on my part. I apologize for that.

Oh, piddle. That came out in not the right way. I'm sorry about that.

Oh, well, it's too late now, it's on the record. And my slide just went away.

So what is worth taking a minute to talk about is what it is that we need. Okay. We, at the end for the day -- what happened to the computer?

DR. PORTIER: I think when you close it, it --

(Pause.)

DR. LOWIT: Okay. Don't touch.

DR. PORTIER: Don't stress.

DR. LOWIT: Don't stress. Right. I'll check my cort levels.

This would be acute or chronic, I don't know. It was atrazine, it's both I guess. What we need at the end of the day is science had informed some difficult decisions, and one of the most important ones we have to make this choice about is what we call the point of departure. And what that is is a

value that we'll select, or a series of values for that matter, that represent whether it's a NOEL or LOEL or a benchmark dose, hopefully for precursor event and mode of action for atrazine that represents a level by which we can -- act as a starting point to create a risk assessment that's safe for human health.

And those can get very complicated, especially for something like this, because duration is an issue, and we've heard a lot about acute versus chronic, and I think the grayness between acute and chronic I think is probably more important to us than -- as much as anything right this second because we need a -- we will need a certain amount of clarity around the relationship between the HPA and HPG and the clarity of those key events to get a handle on how often we need to monitor and water. So whether it's days or weeks or months or years, that kind of resolution is important to us.

Life stage is also important,

whether it's adults, it's pregnant females, it's newborns, it's in utero, it's postnatal, it's perinatal, it's pubertal for lack of a -- it's a route. We look at point of departure by route. I think for atrazine route is less important because we're really in this analysis looking at oral exposure and drinking water. But for a lot of other assessments we think about things in other routes.

The other pieces that we need will be, since we'll be determining a point of departure from animal data, we'll have to extrapolate from animals to humans and that's how you will go from one of those steps that Dr. Pastoor talked about, one of those orders of magnitude to go from the animal value down to a human equivalent value. There are different ways of doing that. The standard way is to divide by 10. There are more sophisticated approaches it can be upon.

From that point we go what we think of as the average human to a sensitive

human, and standard procedure will be to divide by another 10. There are also most sophisticated ways of doing that, that could be anything from something smaller to something far larger.

Specific to the Pesticides Office, since we work under the Food Quality Protection Act, Congress mandates that we add a 10X factor for the protection of infants and kids, and that value is only removed with sufficient data on hazard and exposure.

And so the default window of uncertainties is 1,000. It can be bigger or it can smaller depending on the situation, depending on what science informs the animal to human, the human sensitivity, or the FQPA. So you may start with a default of 1,000, bigger or smaller, depending on the science.

Drinking water, Dr. Pastoor had a series of box and whisker plots, and I believe there quite a bit of comments from Dr. Young wondering about the differences in the --

looking at the totality of the data set versus individual sites. And I think our message tomorrow will be very clear that we assess risk to individuals at individual sites because every site has its own atrazine level and we have to make sure that the people in those sites are protected as individuals.

DR. THURMAN: And this is Nelson Thurman. Just to give you an idea of how we do the drinking water component part of it, because we know we have to -- the temporal and spatial variability we talked to you about, we have to account for that. We know we're not going to get coverage everywhere, over all dates.

So what we end up doing is focusing on what we think are the most vulnerable areas, or the most vulnerable systems. A lot of times that's done based on something like a WARP model, something like -- based on our best information we have based on the relative -- on the crops being grown, the

relative vulnerability of the sites, the weather patterns. Sometimes in the case of atrazine, it's based on the fact that we do have Safe Drinking Water Act monitoring over time that tells us -- helps us zoom in on where we think the most vulnerable systems are going to be.

The goal is, if we can protect those systems, we're protecting the less vulnerable systems. We don't go into this saying that all systems are in trouble. We know that there -- that we're zooming in on the most vulnerable, and the idea is that's a sensitive sup-population for us, and so we want to make sure that we're safe on that level.

We also need to account for that temporal variability over time. And we need to be able to match that to the hazard assessment. So that's what we try to do. A lot of cases we use deterministic models because that's the one we have. In the case

of atrazine we do have a wealth of monitoring data. With that sampling, as I pointed out yesterday, even interpreting monitoring data is a model.

And so we need to account for what kind of uncertainty do we get in those sampling frequencies so that we make sure that we're not saying, This is safe, whenever the actual exposures would be greater. That's our biggest concern in that regard, so, and that's why the questions we have before you on drinking water, the intent is for you to help us figure how best to quantify that uncertainty in sampling frequencies and how we deal with if that duration exposure changes so that we can then go back and determine on those individual systems are they safe or do we have things we need to do.

DR. LOWIT: Okay. So just to sort of close this out, and how those -- how all these pieces are matched, it gets very tedious and complicated very quickly, particularly as

you think about multiple durations and multiple life stages and many different CWSs, of different levels all over the country that -- how those values will be matched.

That you'll match the points of departure from the appropriate duration from the most sensitive life stage, to the -- from the right routes, to individuals that match those profiles in that drinking water area. So if it's an infant, you'll match it an infant water consumption; if it's a adult, you match it to the adult consumption, and that's done for every individual CWS. And as Nelson mentioned, across the country the amount of atrazine in the water varies greatly.

So to try to bullet what those values and those margins of exposure are in three bullets is -- doesn't necessarily provide the fullness and the robustness of what the risk picture looks like, because it varies a great deal across the country.

Oh, and the last point is that

it's less important to think about those margins of exposure for the 2003 risk assessment because we've gone into this analysis with a clean slate. That's the last risk assessment. We're going into this with a clean slate to ensure that everyone around the country is protected.

DR. WILLIAMS: I think -- yes, that was pretty much what I needed, not what I asked for. The only question I have is how do you -- back to Dr. O'Bryne's question -- that the very low doses that were going down from these what seem to be considered kind of high-dose treatments of rats and so forth, how do we know that these low-dose exposures themselves do no have long term chronic effect. Has that been looked at, is it being looked at, or is that just out of the realm of this handle and shouldn't be considered?

DR. LOWIT: Well, to the extent there is -- there are chronic long term rat studies, and certainly the mammary tumors were

a topic that Syngenta covered quite a bit yesterday, so there is -- there are a series of chronic studies in rats. There is the standard, what we'll call the guideline set of different kinds of toxicities, developmental, repro, cancer, different durations, that sort of thing.

There is also what we're -- one of the things that we will do in the September meeting is to bring in the epidemiology from the human epidemiology literature that has its own challenges. But we'll provide a more full picture of the totality of the toxicity profile I think.

DR. PORTIER: Dr. Young?

DR. YOUNG: Given the nature of these sites and the fact that they're likely to be spread out, does it -- spatial correlation, and I know the emphasis here is temporal, but isn't one of the reasons is the sites are likely so far apart that spatial correlation is questionable?

DR. THURMAN: Yes. You know, that's a question -- it depends on what scale you're looking at, but, yes, I think that spatial correlation on a localized scale is going to be very questionable. I think in general we can identify areas where there's a higher likelihood of high exposures, and that's something we've learned a lot more about as a result of the eco-monitoring that I think we're just starting to say these are things we can starting at those. But more of a -- in classic geospatial sense, that correlation is not as -- it's not going to be easy to find, if it exists.

DR. YOUNG: Yes, because the higher levels are likely a change of mean and not necessarily reflective of a correlation structure once they get that far apart.

DR. PORTIER: Thank you very much.

I think we need to move on to Question 1.4.

DR. LOWIT: Dr. Cooper can read.

DR. COOPER: This must be another bad question.

DR. PORTIER: And actually it doesn't even look like a question, it's just please comment.

DR. COOPER: Yes. Charge Question 1.4, based on a review of in vitro studies evaluating the effects of atrazine on estrogen production, the Agency has preliminarily concluded that atrazine does not have a direct effect on the catalytic activity of aromatase. However, with continued exposure, atrazine can cause increased estrone and estradiol production the H295R and JEG-3 cell lines, Sanderson, et al., 2000; Laville 2006; Higley, et al., 2010.

These changes in estrogen production have been associated with increased cyclic AMP, and CYP19, and message RNA, Sanderson, et al., 2000, 2001, that are part of a complex mode of action through which atrazine up-regulates the gene expression of

aromatase and possibly other enzymes within the steroidogenic pathway, Section 3.3.2.3 and Figure 3, Arrow 7 on the draft issue paper. Wow.

The hypothesis that atrazine alters multiple steroids is supported by the increased testosterone concentrations in H295R cells following exposure to atrazine, Higley, et al., 2010, and in vitro studies demonstrating that atrazine affects a number of signal transduction pathways and/or transcription factors in a variety of cells Suzawa and Ingraham 2008; Albanito, et al., 2008.

Up-regulation of the expression of a group of major steroidogenic genes has been observed in JEG-3 cells, Susaw and Ingraham, 2008. In vivo, there is little evidence that atrazine alters aromatase, per se, Modic 2004, and the evidence associating atrazine exposure to increases in serum estrogens appears to be related to a general increase in gonadal and

adrenal progesterone and androstenedione, Modic 2004; Laws 2009.

Where is the question mark?

Please comment on the extent that the available data do or do not support the Agency's preliminary conclusion that atrazine has a general stimulatory effect on steroidogenesis, as opposed to a direct effect on aromatase.

DR. PORTIER: Dr. LeBlanc, your comments.

DR. LeBLANC: Thank you.

I really don't like reading verbatim to an audience, but I have a lot of factual information here, so I am going to read, and please bear with me. But the enzyme aromatase is a product of the CYP19 gene and it's responsible for the aromatization of androgenic C19 steroids to estrogens, 17-beta-estradiol, estrone, et cetera.

Aromatase is expressed in various tissues in both males and females. Gonadal

expression of aromatase is largely responsible for elevated plasma levels of estradiol and related estrogenic hormones in reproductively mature females. Aromatase is also expressed in other tissues, however, including bone, brain, placenta, and others, in males, immature animals. In reproductively senescent females, plasma estrogen levels were likely the product of aromatase activity in these other tissues.

Aromatase gene expression in these different tissues is under different regulatory controls. The tissue-specific regulation stems from the existence of multiple promoter regions on the gene, the CYP19 gene, that control the expression and the differential production of estrogens in response to different stimulatory factors, hormones, et cetera.

In the ovary, aromatase is under the regulatory control of gonadotropin follicle stimulating hormone, FSH. FSH is

produced by the pituitary gland, it stimulates a signal transduction cascade within the ovary, the granulosa cells, resulting in intra-cellular elevation of cyclic AMP levels. The elevated cyclic AMP levels then stimulate the association of the transcription factors SF1 and CREB to their adjacent response elements resulting in stimulation of the CYP19 gene expression.

So in this situation we have two adjacent response elements and there are two factors that are involved, SF1 and CREB. CREB is phosphorylated and apparently both of these transcription factors need to bind, and in doing so then recruit other players that stimulate transcription of the aromatase gene.

Adipose aromatase appears to be under the joint regulatory control of cytokines, including TNF-alpha, IL-6, IL-11, it's under the influence of glucocorticoid hormones, as well as the transcription factor SP1. So there are multiple players in

regulating the expression of aromatase in the adipose tissue. The cytokines stimulate transduction pathways within the adipose sites that result in the interaction of the transcription factor STAT3 and possibly others with their response elements.

Glucocorticoid hormones will bind to their glucocorticoid receptors and bind -- this complex then binds to the glucocorticoid response element to regulate gene transcription. The glucocorticoid receptor -- I'm sorry, SP1, which is activated by a phosphorylation event, also binds to a response element within the motor region of CYP gene to regulate gene transcription.

Together these transcriptional regulators stimulate aromatase activity in the adipose tissue, and, again, these factors don't seem to function independently in regulating transcription, but seem to cooperate in the regulation of transcription in adipose tissue. Thus, chemicals that

modulate any of these pathways could potentially impact aromatase expression.

Finally, in the placenta, aromatase appears again to be under multiple regulatory controls, and there's much less known about the regulation of aromatase in the placenta, but perhaps most relevant to our discussions is that ligands to the retinoid X receptor, RXR, appear to be involved. And accordingly, compounds such as retinoids, fatty acids, as well as some xenobiotics could influence aromatase activity via this regulatory pathway.

Now we know that atrazine has been shown to elevate aromatase activity in cultured cells, and at issue here is whether or not this is a specific affect of atrazine on aromatase, or some generalized affect on steroidogenesis. In terms of atrazine directly impacting the catalytic activity of the aromatase enzyme, I'm aware of no precedent for this in the literature. I'm

furthermore not familiar with any precedent of the aromatase enzyme being susceptible to allosteric interactions, et cetera, that would result in some direct increase in catalytic activity through interacting with a ligand.

As discussed above, there are multiple regulatory elements through which atrazine could impact the expression of the CYP19 gene, and the greatest evidence that exists is interaction involving the cyclic AMP SF1 pathway. Atrazine has been shown to increase cyclic AMP levels in cells, and it has also been shown to activate SF1 via increased phosphorylation.

There is one paper that indicates that atrazine actually binds to SF1 and activates, but that's been discounted in at least one other paper, and I think the argument against that is rather good. So I don't argue that SF1 may be activated, but I don't think it's through direct interaction with the atrazine. I think it's through

perhaps a phosphorylation event.

Now, at it turns out, SF1 is not a -- the signaling pathway is not a specific regulatory process for aromatase. SF1 is recognized as generally stimulating steroidogenesis in cells, and so it may very well be that this effect of atrazine on the SF1 signaling pathway is indeed a general effect on the steroidogenic pathway and not specifically on aromatase. And I say that with caution because I'm not trying to imply that it's a non-specific effect. It is a specific effect but involves multiple player in steroidogenesis.

So the conclusion can be made that atrazine's effects on aromatase gene expression represents a generalized effect on steroidogenesis, probably mediated by SF1 and cyclic AMP. Potential interactions of atrazine via other known regulatory sites, however, must be evaluated. There are a lot of potential regulatory pathways through which

atrazine could be acting, and they haven't received a lot of attention. And I think we need to consider that.

For example, the glucocorticoid hormone pathway. It has been evaluated with respect to whether or not atrazine binds to glucocorticoid receptor, and the evidence indicates that it doesn't. However, we do know that atrazine stimulates glucocorticoid levels, so in that respect we could be inducing endogenous glucocorticoid levels with atrazine treatment that is resulting in a stimulation of aromatase via the glucocorticoid hormone pathway that can't be excluded at this point.

SP1 was another transcription factor involved, and I could find no evidence that atrazine has an effect on SP1. The information simply isn't out there to make a judgment one way or another. The same thing for RXR. I identified RXR as a potential target for atrazine, but at this point in

time, as best that I can determine, there's no evidence to indicate whether or not atrazine is binding and activating the RXR.

So in conclusion, there's significant evidence that atrazine has the potential to regular gonadal CYP19 expression through the stimulation of the cyclic AMP SF1 signaling pathway. This effect would be expected to be common to most sterogenic pathways along the hypothalamic, pituitary, adrenal, or gonadal axis. It functions on both axes.

It's also plausible that atrazine stimulates adipose CYP19 expression through its activation of the HPA axis resulting from the increased glucocorticoid levels that we see with atrazine treatment. And that's all.

DR. PORTIER: Thank you.

Associate discussant Dr. Delclos.

DR. DELCLOS: I won't have that much to say. I think that, as indicated in the document, as Dr. LeBlanc has said, there's

clear evidence that the ATR do not directly interact with aromatase, but that the enzyme is induced after longer term exposure in several cell lines in vitro. And one thing that struck me in reading the literature, and was pointed out in the public comments yesterday in the Syngenta, is that most of these in vitro studies take -- do not take into account the metabolism and the relationship of -- they use atrazine, they don't take into the account the metabolic capabilities of the -- in the animal and what happens and it seems like the metabolites are really the major players after atrazine gets in the body.

But that being said, there are a couple of studies that did use the metabolites and they found that the major metabolite, DACT, is not active, but the two monodealkylated metabolites, DIA and DEA, are active, although they may be a little less potent than ATR itself. So there is some

relevance of these in vitro activation studies perhaps.

But there is little evidence in the rodent studies, or there's no direct evidence in the rodent studies that aromatase is being elevated in vivo. The only evidence that's been used to support that is the pattern of hormone stimulation and reduction in the testosterone elevation of estradiol and estrone. The studies that have directly looked at aromatase elevation, the Modic thesis that was presented, and the rest, and the Sanderson abstract, showed no evidence that gonadal aromatase is induced in vivo after atrazine exposure.

Several possible other explanations for the elevation of estradiol and estrone have been presented, including increase for the aromatizable androgenic substrates effects on metabolism of the estradiol, or induction of adipose aromatase, and I think that hasn't been worked out.

But there is some evidence from the in vitro studies, the study by Higley that was cited, that there is a general activation. And as Dr. LeBlanc talked -- said, there are multiple pathways that have been proposed to be affected by atrazine or its metabolites that may support a general induction of steroid synthesis.

So I think that there is no -- to summarize, there is no existing evidence in the rodent studies that there's an elevation in vivo, and there is supporting evidence that there's a general increase in steroidogenesis, so I think the hypothesis is supported, although it's not definitively proven; there's still work to be done in that regard.

DR. PORTIER: Thank you.

Dr. Horton?

DR. HORTON: Thank you.

Just a reminder that when you're talking about steroidogenesis in vivo, and particularly when you're talking about the

ovary and testes, you're talking about a very complex organ. Steroidogenesis in the ovary is a two-cell process that requires the interaction between the fecal and granulosis cell, and you have to have enzymes in two different cells working together. You also require the interaction between two gonadotropin in order to make the ovary work properly, and those two gonadotropin must be secreted in sequence in order to have the ovary work properly in its cyclic fashion.

And what I've not heard discussed is the role of atrazine or any impact of atrazine on the secretion of follicle stimulating hormone. I've heard a great deal of discussion of the role of atrazine on the secretion of luteinizing hormone. So what's missing from this discussion is the potential impact of the set up of the ovary and its preparation for steroidogenesis by follicle stimulating hormone, because in an ovary that is not prepared properly, it will not respond

to luteinizing hormone in the appropriate way.

So I would encourage any studies to -- in the future to also examine the levels of follicle stimulating hormone. Thank you.

DR. PORTIER: Dr. Williams.

DR. COOPER: Can I just make one comment?

DR. PORTIER: Sure.

DR. COOPER: FSH has been looked at in the ovariectomized estrogen primed female and in the male, and there hasn't been really any change noted in that they're relatively short term treatments. That's just the background that somehow slipped. I apologize you didn't see that.

DR. PORTIER: That was Dr. Cooper, EPA.

Dr. Williams?

DR. WILLIAMS: I would say I don't have anything to add to what's already come out. I agree with the comments that have been made.

DR. PORTIER: Dr. Schlenk?

DR. SCHLENK: Yes. One of the things we've dealt with a lot with -- in terms of pesticide metabolism, is that with drug interactions, oftentimes when you see issues related to clearance, it's not necessarily induction of pathways, it's actually inhibition of pathways. They tend to be more thermodynamically sort of, you know, favored, if you will.

One of the things I didn't see in the White Paper, and has not been mentioned all that much, are the inhibition of catabolic pathways that atrazine may actually take -- have some impairment on. And I know I think we talked earlier, CYP3A is actually inhibited fairly potently by atrazine, at least in about a one to two micromolar, and I've got a reference to throw in the report.

But one could argue that the enhancement of the steroid levels perhaps would be due to inhibition of catabolism

perhaps, of steroid -- rather than looking at the steroidogenic pathway, but actually looking at the catabolic pathway that actually keeps the levels high. And honestly, thermodynamically that would make a little bit more sense perhaps. But it may be, again, a minor pathway outside of what the hormonal -- luteinizing hormone issue is related to that. Just something to consider.

DR. PORTIER: Additional comments?

(No response.)

DR. PORTIER: I won't even attempt to summarize this. I'm assuming Dr. LeBlanc's got it all down and, well, his comments were pretty clear, if you could follow it. Which I'm sure Dr. Cooper could; maybe not the rest of us.

I think the general conclusion was that -- let's see if I can -- clear evidence it's not direct. That's what I got out of this. There's a lot of evidence for indirect, but not a lot of evidence for direct effects.

Okay. Why don't we move on to Question 1.5 then.

DR. COOPER: Charge Question 1.5, a series of studies evaluating the effect of either peripubertal or gestational exposure to atrazine in the male rat indicate that atrazine decreases testosterone concentrations and may lower androgen dependent tissue weights, but that it has little effect on basal LH secretion in the male. This response generally occurs at doses of 50 milligrams per kilogram and above.

In contrast, atrazine in vitro has been found to consistently increase steroid hormone production. Thus, there is an apparent discrepancy between the in vivo and in vitro data. However, our understanding of the interplay between the adrenal and the gonadal axis may provide an explanation -- period. An explanation.

(Laughter.)

DR. COOPER: At the doses used in

the in vivo studies, atrazine induces an activation of the adrenal axis, increased ACTH and corticosterone, and the increase in corticosterone does not habituate, i.e. it does not lessen with repeated dosing. There are a number of studies in the literature demonstrating that prolonged corticosterone stimulation will impair testosterone production by a direct action on steroidogenesis in Leydig cells.

Therefore, in vivo effects of testosterone synthesis are likely the result of an adrenal hormone-mediated down-regulation of the HPG axis through corticotropin releasing hormone, CRH modulation, or GnRH pulsatility, or directly in the testes through a glucocorticoid receptor-mediated change in steroidogenesis.

Please comment on the degree to which the proposed mode of action, Figure 3 of the draft issue paper, provides a biologically plausible explanation for the decrease in

serum testicular testosterone identified in the in vivo studies. Please comment on the extent that the available data do or do not support this hypothesis.

DR. PORTIER: Dr. Delclos is the lead discussant.

DR. DELCLOS: Okay. Well, the white paper reviews several studies in which testosterone levels are replaced by exposure to atrazine during the peripubertal or gestational period, and at least one study where exposure to commercial formulation involving atrazine in inerts in adults reduced testosterone, and there was generally no consistent corresponding effect on LH, although there was evidence of a decreasing trend in at least one study, the Stoker study.

There was no direct assessment of the HPA axis in these studies, but the proposed MOA involving HPA axis is certainly plausible, given the known suppressive effect of corticosterone and corticosterol -- or

cortisol on the HPG axis at all levels, and the clear evidence that, from other studies, that atrazine can stimulate the HPA axis at levels at which the testosterone reduction has been reported. So there is suggestive evidence supporting this MOA.

But there are a few issues. As I said on the first day in my question, I'm intrigued by this fact that the major metabolite, DACT, affects puberty, which is a sensitive short term endpoint, but does not affect, or only weakly affects, the HPA axis. And the pubertal delay can occur at levels below those at which ACH, cortisol increase, the corticosterone increases have been demonstrated. Presumably this affect on the HPG axis is important in this delay in the -- in puberty in the males.

And at least in the one -- in the study of the male, the PK study by Ross, which is the only one that I'm aware of that looked at specific metabolites in various tissues,

but they did find high persistent levels of DACT in the brain. So I think this is just something that could be -- should be further evaluated.

The other issue was the Rosenberg study which indicated that prenatal exposure led to a lasting affect on testosterone and delayed preputial separation, and there was no data on the HPA axis provided from that exposure, but where expected activation in the dam was likely.

I'm not really too aware of the effects of activation of HPA axis on puberty. I know that there's at least one study looking at I think dexamethasone to the dam indicating that puberty can be delayed, and there was a paper last year from Richard Sharp's lab in which he was -- looked at the affect of dexamethasone on dibutyl -- interaction with dibutyl phthalate in reduction of testosterone, and he had indicated that there was no effect of dexamethasone on the --

directly on the testosterone levels. There was a decreased birth weight in the neonatal -- in the pups, but that it greatly enhanced the ability of dibutyl phthalate to reduce testosterone synthesis.

So, again, this is -- these are just the, as I say, the proposed mode of action is plausible, but I think it needs to be further evaluated in these -- particularly in the pubertal exposures and the gestational exposure.

DR. PORTIER: Dr. LeBlanc?

DR. LeBLANC: Ample evidence indicates that atrazine stimulates the hypothalamic-pituitary-adrenal axis resulting in increased production of adrenal corticosteroids and progesterone. Induction of HPA axis is well known to suppress the HPG axis predominantly through the inhibition of hypothalamic GnRH and/or the secretion of gonadotropin inhibiting hormone resulting in the suppression of LH from the pituitary.

And I find this proposed mechanism for the suppressive action of atrazine on testosterone production largely plausible. There are issues that I still struggle with, and I'll try and communicate right now. One is that -- well, we had this issue of discrepancy between in vitro and in vivo observations, and it seems to me that the weight of the evidence is indicating that in vitro we're looking at the perturbation of the axis, and that's perfectly plausible.

And it appears that in vitro we're seeing effects, at least on aromatase, via some direct regulatory pathway within the cells. And right now SF1, cyclic AMP seems to be the -- there's the most evidence for and interaction at that level. However, and so the presumption would be that both of these processes would take place in vivo, but I suppose the effects on the axes perhaps predominate and so we're not seeing the induction of aromatase in vivo, or it might be

a consequence of pharmacokinetics of atrazine. I certainly don't know the answer to that.

However, the pituitary also is regulated by SF signaling, specifically LH production -- SF1 signaling. I'm sorry. And so if we accept the premise that SF1 is having a stimulatory affect, it certainly raises issues as to why we're not seeing a stimulatory effect of the activation of SF1 at the level for the pituitary, which would result in an enhanced steroidogenesis. I'm not sure.

The other thing I struggle with is if we're looking at an overall -- let me think about this. I'm going to stop there. I'm going to get myself too deep into a hole here that I'm not going to be able to get out of --

(Laughter.)

DR. LeBLANC -- and I don't think I'll contribute anything of significance. So I'll stop there.

DR. PORTIER: Dr. O'Byrne.

DR. O'BYRNE: I just have one comment to make. In the context of decreasing luteinizing hormone, I see it time and time again, it's difficult to see a reduction in gonadal intact animals. That doesn't mean to say there isn't a decrease because it's below the detection of the assay, and that is something that really has to be taken on board. So when people say there's no decrease, I don't believe it. And that's all.

DR. PORTIER: Dr. Selvage?

DR. SELVAGE: From what I've heard, I don't think I can really add much more to this. So I will pass.

DR. PORTIER: Dr. Williams.

DR. WILLIAMS: The only thing I would add is the -- in thinking about kind of peripubertal or gestational exposures, what really hasn't come out -- we've talked about direct effects, but thinking about kind of epigenetic changes that are going to later impact on many different systems.

So, you know, the cell culture evidence I think it's pretty straightforward and easier to interpret. But the in vivo data I think is going to take a lot of mechanistic examination before we can really clearly put a story together, which I think is what Dr. LeBlanc was getting at. But it's going to be really complicated. And if you, you know, directly answer the questions here, you know, there's a general stimulatory effect. I mean that seems rather superficial when you consider what might really be going on from a neuroendocrine standpoint.

DR. PORTIER: Additional comments from the panel?

(No response.)

DR. PORTIER: So what I read from this is that the -- the mechanism of action is plausible, all right, but really needs more -- at least the in vivo is plausible but needs more explanation, and the in vitro stuff is what it is. I mean you believe the in vitro

stuff, but the in vivo, more mechanistic information to really be able to interpret why it tends to conflict. Is that a clear summary?

(No response.)

DR. PORTIER: Additional comments?

(No response.)

DR. PORTIER: I'm not sure that helps, but --

(Laughter.)

DR. PORTIER: I want to give Dr. LeBlanc a few more minutes to think through this. I can just see the wheels turning. He's thinking, Well, maybe I'll get this figured out by lunch.

I don't think we're going to get much more from the panel on this.

Dr. Lowit?

DR. LOWIT: Even with the lack of clarity I think we heard what we were looking for.

DR. PORTIER: Dr. LeBlanc.

DR. LeBLANC: Just one comment. I think one of the reasons, at least one of the reasons that I struggle with this, and I'm not as close to the data as Dr. Cooper is, but it seems to me, with respect to steroid hormone levels, depending upon which study you're looking at, different things happen. Sometimes things go up, sometimes things go down, sometimes they don't change. And so, you know, I don't think we can explain that right now, but it's also hard to come up with a biologically plausible explanation for what's going on.

I mean, does the weight of the data indicate that testosterone is going down?

DR. COOPER: There's several different studies and several different dosing regimens, but as the background paragraph said in this thing -- it did a good job of capturing that anyway, I think -- is that the studies that have been done generally imply there's a decrease in T, but they're, you

know, they're in vivo, longer dosing, and that's that disconnect between the in vitro and the in vivo studies.

I will add that there is evidence that there's an increase in testosterone in Leydig cell preparations. And that was done -- increase in testosterone production --

DR. LeBLANC: In the cells but not in the plasma?

DR. COOPER: In vitro, the --

DR. LeBLANC: Oh, in vitro.

DR. COOPER -- in purified Leydig cell preparation, which is another -- those studies were done as a part of the EPA's Endocrine Disruptance Screening Testing Program's development of the H295R cells, whereas background work that was done in those -- this isn't published, so we couldn't lay it out there, but I think for clarification the studies that have been done, they were comparing directly Leydig cells against H295R to see how representative the

295R were of the "real thing," rat Leydig cells.

And the data that has been obtained so far -- and, again, these are different labs that ran these studies -- showed that -- and they were looking at the production of testosterone and estradiol -- that not only did estradiol or estrone, whichever your measurement -- your estrogen you wanted to measure, were increased in response to atrazine. Again, going back to the Sanderson work and one of the primary reasons they selected that cell line, but also the testosterone increased.

And the Higley paper points to that H295R data, and as a part of the supporting validation of that assay, the Leydig cells were compared, and in the Leydig cells they saw increased T. That's the in vitro evidence that it's increased steroidogenesis more than just estradiol, and in addition other hormones were looked at and

they were up.

And then there's this acute increase in testosterone that you'll almost always see when you look in vivo, but the chronic it's always decreased, and that's what's the disconnect that we were seeking guidance on, which we probably confused you more than we're confused.

DR. PORTIER: I don't think we saw any additional insight. That's -- if I could -- if I heard that.

Any additional comments?

(No response.)

DR. PORTIER: I'm going to leave this question open because I really do see the wheels turning. I suspect he'll come back from lunch with a light bulb over his head.

Why don't we -- we're going to move on to the next question, 1.6. I don't think we'll finish it before we break for lunch, but we can begin the discussion on that question. And it may take us 15 minutes just

to read the question.

DR. LOWIT: We're going to do a quick musical chairs.

DR. PORTIER: Bringing in the neurotox expert. Right?

DR. LICCIONE: I was hoping Ralph Cooper would read this because he's had a lot of practice today, but I'll try to read slow, I'm not as experienced as he is.

Based on a review of the neurotoxicity studies, Section 3.4 of the draft issue paper, the Agency has preliminarily concluded that several recent studies provided further support for the concern that dopaminergic neurotransmission may be affected by atrazine, a concern that was raised by studies prior to 2003.

However, the Agency has concluded that several aspects of these studies of the dopaminergic neuronal pathways, in particular the changes noted in the stereological evaluations and the observed changes in

behavior, should be considered as preliminary findings. And in my presentation I also emphasize inconclusive.

This conclusion is based on the notable limitations identified in the data including, one, a lack of clear dose-response relationships; two, lack of inclusion of suitable positive controls to confirm the competency and reliability of the procedures utilized in examining dopaminergic systems in the brain; three, limited data to corroborate stereological findings; four, limited or no supporting histological and behavioral assessments; and, five, no consideration of the potential role of the HPA axis, for example, alterations in corticosterone.

EPA has further determined that two non-dompaminergic neurotoxicity studies, one on brain somatostatinergic systems and the other on neurobehavior in mice, also have significant limitations. For example, the lack of details on the source and purity of

atrazine; the age and body weights of the mice; poor quality of the amino cupric silver staining photomicrographs; limited presentation of the reverse-transcriptase polymerase chain results; lack of data to corroborate the conclusion of neuronal degeneration; reference citations did not support statements made in the text; no explanation of biological plausibility of the alterations in somatostatinergic receptor sub-types; inappropriate statistical evaluation of data, litter versus the pup; discrepancies in the number of pups examined for behavioral endpoints; and lack of objective and validated behavioral tests. Please comment on these preliminary conclusions regarding the neurotoxicity findings.

Okay. The Agency has preliminarily concluded that the available studies indicate that the neurotoxicity endpoints examined are not more sensitive than those evaluated for neuroendocrine function

following atrazine exposure. For example, attenuation of the LH surge and estrous cycle disruptions in female Sprague-Dawley rats which form the basis for the current chronic RfD.

In addition, the Agency has concluded that there is no association between atrazine exposure and development of Parkinson's disease on the basis of non-specificity of effects on brain dopaminergic system, lack of histological and behavioral features characteristic of Parkinson's disease, and results of epidemiological studies. Please comment on this preliminary conclusion.

DR. PORTIER: Thank you.

So, Dr. Horton, it looks like we have two comments we're looking for. And turn on your mike, please.

DR. HORTON: Let's see if I managed to respond to both of those. Okay. In response to the first comment, the Agency

provides information in Section 3.4 of the white paper and the presentation given on Monday, that there reason for concern that dopaminergic transmission may be affected by atrazine, as shown by studies conducted prior to 2003. The Agency then reviews two more recent studies, Belloni, et al., and Coban and Filipov 2007, which provide additional data reaching similar conclusions.

In addition to previous suggestions that atrazine may negatively impact dopamine transmission, the Coban and Filipov study also suggests that serotonergic systems may be involved. Additional studies were presented suggesting that atrazine may also influence somatostatinergic systems as well as other neuropeptides. Any alteration in these neural pathways may ramify through many homeostatic, neurobehavioral, and metabolic systems, as mentioned in the response to Question 1.2.

Although each study individually

suffers from technical errors and problems with reporting and should be considered preliminary as is consistent with your conclusions -- or the conclusions of the Agency, when considered in total, the emerging body of evidence suggest an area of concern that requires further investigation into the neurotoxicity and neuroendocrine effects of atrazine across all stages of a life span in environmentally relevant doses.

These areas of concern include the prenatal/perinatal periods and effects in adulthood on a wide range of neurotransmitter systems should also include the oft neglected, but nevertheless important glia, and bring regions other than those defined by a strictly Parkinson's disease model. Research should be conducted with consideration to the fact that there are critical periods during development, both prenatal and postnatal, including during the adolescent period -- see work by Cheryl Sisk and others, and I'll provide a list of

references -- when transient exposure to chemicals can have permanent organizational effects on the nervous system.

Additionally, cumulative exposures to steroids and/or stress is proposed to have cumulative or allostatic affects on health, and I can provide references. There's a recent reference that has just appeared in the general Hormones and Behavior by Bruce McEwan and John Wingfield. That has just appeared in Hormones and Behavior 2010.

Of concern to human health and well-being is the suggestion that atrazine may affect numerous regions and neurotransmitter systems. Careful wording must be used when reporting these new developments. Use of the phrase non-specific, as with a non-specific or non-Parkinsonian model, conveys a sense of unimportance. In contrast, the possibility that multiple neurotransmitter systems are involved, in fact, should shift our focus on the mode of action.

The early studies were focused on a model fo Parkinson's Disease. The realities of current research funding mechanisms often force researchers to squeeze their work into the context of one disease model or another, whether the square peg fits in the round hole or not. With the emergence of more research, the peg may not fit, and appears not to fit the Parkinson's disease model, but now needs a new hole of its own.

The emerging preliminary data do suggest a plausible link and basis for additional studies related to the new mode of action for atrazine proposed in the Agency's white paper. Additional research is needed to determine the size and shape of the new peg and the new hole, including studies specifically designed to evaluate the changes in neurotransmitter and neuropeptide systems in -- concerned in the context of the broader mechanism of action proposed in the white paper.

For the record, researchers working with animals face increasing limitations on their ability to meet the criteria set forth by the EPA for determining whether data are acceptable. Researchers are under increasing pressure to limit the number of animals used in their work, and one question they must respond to on every animal care and use regulatory form is whether or not an experiment replicates an experiment conducted by their laboratory or any other laboratory or any published study.

It is increasingly difficult to get permission to replicate a study that has been done in the past, and to get permission to conduct that study from your institutional review board. Any attempt to replicate results -- or any attempt to replicate results of a previous study must be done in the context of new studies, thereby increasing the complexity of an experiment, thereby often weakening your statistical power.

We encourage the EPA to communicate with the National Institutes of Health, the Department of Agriculture, the National Science Foundation, and the American Association for Laboratory Animal Care, the accreditation agency for animal facilities, to the extent possible to make them aware of the EPA's guidelines for acceptable data. Thank you.

DR. PORTIER: Thank you.

Dr. Chambers.

DR. CHAMBERS: Thanks.

I think points are well taken, but I think I'm going to take a different tack on answering this question. I think a number of people around the table today have mentioned the dose levels that have been used in a lot of these studies, and I'd just like to reiterate my concern about some of those dose levels too.

Necessarily, in academic labs I think we need to go to high doses just to sort

of justify that we get some results that we can publish, but that doesn't necessarily do you a whole lot of good in the regulatory arena, and I'd like to emphasize that.

Another point along those lines too, is when you start using these astronomical doses that we've seen numerous times in these studies, you pretty much obliterate the metabolism in other sorts of defense mechanisms, and so they're not necessarily realistic. So the mode of action you discover at those high doses may not be anywhere near reality.

With respect to your first question there, I think -- I looked at all those studies and I concur with the deficiencies that you identified, and so I don't see that many of them are very useful for risk assessment. They certainly are not designed and not done to the quality that you expect out of the registrants and the other types of studies that are used in the

regulatory sense.

Just for, I guess, the sake of transparency, I do want to mention that the Filipov studies, and there are three of them, did come out of a center grant that I was in charge of at the time. I did not collaborate on those studies; I had nothing to do with them. As a matter of fact, I did comment on the high doses and did object to those at the time, but they got done anyway. But at any rate, I don't -- I thought maybe I better say that just in case anybody knows where Filipov was at the time that he did those studies.

So you identified a number of rather non-specific effects -- oh, I shouldn't use non-specific I guess -- sort of scattered effects, non-dose responses, scattered types of things, and I don't think they really reflect a strong indication of neurotoxicity. Probably the study that is most useful from the standpoint of identifying whether any of these neurotoxic mechanisms on dopamine are

any more relevant to your point of departure in risk assessment would be the Rodriguez study, and I still have difficulty with the way they tried to describe dose levels there.

And, Ruby, I tried to interpret that the way you did yesterday, too, but I looked again last night, and every time where they talk about the chronic dose levels in the feed, they just say five mgs per kg or ten mgs per kg and don't identify day. And I see in your write-up you identified day, and that's sort of the interpretation. But nevertheless, that is not the way you express chronic doses, and so it was really very difficult to tell what they were being exposed to.

They should have described, for something like that, a feeding study, was this put in pellets, was this powdered form, and, you know, did the animals scatter it and did they really understand how they were getting it, is it something they put on cookies, like sometimes we do with feeding studies and so

forth? And so I think you're really left with not knowing for sure how they animals were dosed and how consistent that five or ten mgs per kg per day, I presume, was done.

I'm concerned -- so that probably is some of the lowest dose data that's in this mix. I'm rather concerned about the stereological results because those have a tremendous amount of overlap in the individual animals between the treated and the control. So I'm not really convinced that there's an effect there. There was a limited number of animals that are presented and the -- again, a tremendous overlap between the controls and treated. So if there's anything there, I really do think that needs some sort of repeat some time along the line.

I'm not sure what else to comment on that except, again, these are not data that are consistent enough and well-described enough for you to conclude anything other than that they are preliminary and they have

limitations.

With respect to the Parkinson's disease question, I think the studies that have implicated Parkinson's disease there are basically an over-interpretation of the results that they've gotten. I'm not convinced that the studies that they've done, because of the behavioral endpoints and that sort of thing, are really suggestive of an implication in Parkinson's disease. That's not to say that that isn't true, but there's nothing in these studies, I think, that really suggests that.

So I am in concurrence with your evaluation of those papers that they are preliminary, they're non-conclusive, and they probably do not lead to any suggestion that these effects should supplant the POD that you've already got in place.

DR. PORTIER: Dr. Reed.

DR. REED: First of all, I want to thank the Agency for putting all the

literature paper and its collections in such an organized fashion. It really helped to go in -- you know, the summary, the review they're very helpful to guide me in terms of which particular article that I want to zoom in. I did go and get all the articles, so that helps too. And so that, you know -- what I mean is that we don't have to agree with the review or the interpretation of this study, but the guide is great.

Let me see, for the first question, again, my take is going to be slightly different, so that's great. You know, all three of us look at different aspects of the issue. So I also agree that the Agency came to the preliminary conclusion about the findings being preliminary because of limitations.

Basically, when I look at neurotoxicity, I am looking at whether CNS, PNS have been affected, and atrogen technically is neurotoxic. I think what was

puzzling and what all the studies were going about is trying to connect between mode of action and the manifestation of that, which we are not clear about how many different mode of action or networks involved, and so it's sort of at this stage impossible to connect to the two. And we're going to be seeing a lot of that expression within, you know, our meeting here.

I have one comment because I came to the same conclusions as Dr. Chambers that if I were to look at the endpoints, which would have some kind of an impact on risk assessment, it will be the Rodriguez study because of the low, you know, NOEL. And I'm not trying to defame the study at all because I'm not entirely clear about how the dose was decided, I just caught on the fact that it says that they controlled the diet, so I figured that they must have made measurements of the diet in order to control the body weight.

I am impressed with the fact that these are fairly recent studies, and so, you know, yesterday I mentioned with the oncogenicity part of our discussion and in my questions, you know, during the presentation, and also I'm just struck by the fact that if something is important, can we perhaps make an effort to contact the author and maybe clarify some of these points? I mean if we think there is value in it, I would encourage the Agency to do that.

I notice that one of the authors in the Rodriguez study is actually from the Agency. Am I correct on that? Maybe I wasn't. But -- is it? Okay. Well, in any case, that's what I'm thinking, that if it is possible to contact the office, we might be able to get information if dose is the issue.

DR. LICCIONE: I have a comment though about the confidence in the study. I mean I think at face value, as Dr. Chambers mentioned, the stereological results, I have

zero confidence in that. And I have a number of problems with the behaviorial assessments.

DR. REED: Right. Right.

DR. LICCIONE: I believe that in contacting them we would have to address a lot of these limitations --

DR. REED: Right. Right.

DR. LICCIONE -- rather -- in addition to clarifying the dosing regimen, which I also had difficulty understanding because they didn't provide the correct information.

DR. REED: Right. No, no, I wasn't --

DR. LICCIONE: But I think --

DR. PORTIER: That was Dr. Luebke.

Dr. Reed?

DR. LOWIT: Luccione.

DR. PORTIER: Luccione. He got it wrong.

Dr. Lowit.

Okay. Back to Dr. Reed.

DR. REED: No, no, I totally agree with you. I just haven't done with the concept. I'm saying that if it is just a matter of dose, then, you know, we can go and contact, but I think there's some protocol observational issues that we might not be able to resolve is what I was going to say. So that's good. We're all in agreement with each other.

In terms of neurotoxicity, I ask myself what kind of an endpoint am I looking for anyway, and it's going to come up in Question 1.9, but what I'm thinking of, and, you know, it's something sort of -- focused me in the sync, it was because I notice that some of the studies, they might not be listed within this group, actually came up with clinical sign kind of effects, and I don't know the reasons behind it, but I think that that might be something, you know, worth looking into in terms of manifestation of neurotoxicity.

Let me see. I think I would focus on neurobehavioral and neurodevelopmental, which my understanding is that we don't have something like that. I don't want to be the R2D2 type of person as a risk assessor, but it might be worthwhile for the Agency to, instead of saying that we don't have enough information, these are all preliminary, we don't think the endpoint is going to be more sensitive than what we've been looking at, since we haven't defined that so we cannot make such conclusion, it might be worthwhile to look into the possibility of -- and I think the Agency accumulated a lot of great information, asking a lot of good questions about neurotoxicity, that it might be possible to work together in coming up with a neurodevelopmental study, or even FOB type of study, at least explore that possibility. I think it's good to well characterize it before we throw it away saying it's not as sensitive.

DR. CHAMBERS: Let me ask a

question since this is a pesticide, was a DNT study conducted on it? No?

DR. LOWIT: No, there's no DNT.

DR. PORTIER: What does that stand for?

DR. LOWIT: Developmental --

DR. CHAMBERS: Developmental neurotoxicity study.

DR. LOWIT: Sorry.

DR. CHAMBERS: Sorry.

DR. REED: Can I make a comment or question? I don't think there's FOB study. Right? Was there?

DR. MENDEZ: I don't believe so, but I'm not 100 percent sure --

DR. REED: Acute versus chronic or --

DR. MENDEZ: I don't think that we have an acute neurotoxicity study, if that's what you're asking.

DR. REED: And so these are the two, DNT and the neurotoxicity studies is what

I'm referring to.

DR. LOWIT: I guess I'll go back to one of my comments earlier about the -- particularly the DNT that costs somewhere in the order of 7, 800, up to, you know, upwards of a million dollar study, and many, many, many animals. The value added of that study, given the nature of the totality of the -- what is known about atrazine and that we're really diving down into more precursor events, and DNTs, developmental -- you know, the stuff, the alphabet soup -- developmental neurotoxicity studies are not always the most sensitive studies, and it's, at least my personal opinion, it's highly unlikely that study would give you anything lower than the things that we're talking about for the -- related to the hormones.

DR. PORTIER: Dr. O'Byrne?

DR. O'BYRNE: My understanding is that atrazine has been around for 60 years. Surely there's some clear epidemiology -- epi

data because some of these male farmers must be now in their 80s. So is there not a clear guidance from that sort of data, clinical epi data on Parkinson's?

DR. LOWIT: I missed the first half of the question. You're asking about --

DR. O'BYRNE: It's been around for 60 years. Farmers have been spraying it on their crops. And some of those guys now must be in their 80s, so is there is any epi data showing that there's an increased incidence of Parkinson's disease? Do we need animal models?

DR. LOWIT: There is -- our epidemiological evaluation is still ongoing, but there is -- atrazine was included as part of the Agricultural Health Study, and there is a relatively recent study on Parkinson's, and atrazine was not one of the pesticides where they saw a positive statistical association with Parkinson's in pesticide applicators.

DR. PORTIER: Was it Dr. Horton?

Yes. And then Dr. Chambers.

DR. HORTON: One of the things I want to make sure that we made clear is the distinction between neurotoxicity and neurodevelopmental effects. Whereas, you know, we have one killing neurons in an acute fashion, whereas exposure at different stages of life altering a developmental program, and I think those are two very different things that have to be examined, which is why, in my statement, I commented that the studies need to be examining factors at different life stages.

And I think that is some of what needs to be examined in terms of looking at weight of the evidence. And I think looking at the totality of the evidence, regardless of what you're looking at in terms of the doses and things here, there is some suggestive evidence that points in that direction.

The other question I have is for Dr. Liccione -- okay. I'll get it. When you

say you discount the stereological data, part of it was you commented on the calibration. Could you comment -- or explain why you discounted it and which specific study?

DR. LICCIONE: Okay. The Rodriguez in particular --

DR. HORTON: Okay.

DR. LICCIONE -- the stereological showed, as Dr. Chambers said, when you see the overlay from a variability, the stereological results should account for a coefficient of error biological variation to give you a handle of the statistical dispersion. Because stereology is based on random systematic statistical sampling, which is often used in survey sampling, and one has to address those statistical issues in looking at it because stereology has not gained wide acceptance yet because of a lot of problems with methodology and statistical error.

DR. CHAMBERS: This is Janet Chambers. I just wanted to make sure that my

question wasn't interpreted as a suggestion that DNT be run. I was just thinking that perhaps it was already part of your data sets.

But I think sort of following onto that conversation that was going a few minutes ago, I think what you all really need to do is make sure that you're looking at what appears to be most sensitive out there, and not requiring a lot of data that are just probably going to be false leads because they're only go to show effects at higher levels and all.

You've got the data sets that you can look at for the most sensitive effects. I think you pretty much decided that the neuroendocrine appears to be the most sensitive effect right now, and going into a bunch of other types of data requirements or data studies that are not related to the very sensitive effects are probably not going to lead to anything very useful.

DR. PORTIER: My understanding of the question to the panel though is whether

you think the neurobehavioral, neurotox effects are less sensitive than the endocrine.

DR. CHAMBERS: And I think I'll try to answer that again, Ken. Based on these particular papers that we saw, I don't see that they are -- there is anything that is more sensitive than the other endpoints that they've used up till -- that you've used up till now.

DR. PORTIER: But I think we have some -- actually, I think Dr. Reed was next, and then Dr. Horton.

DR. REED: By saying neurodevelopmental effects, I also don't mean the standard -- what I said is that the Agency had gathered a lot of information by now specific to atrazine, but I thought by working together you might be able to come up with a valid -- a study to study the neurodevelopmental effect manifested from -- whether it's, you know, endocrine effects or not, because I think you're going to get a lot

of questions further down the road about what happened to the pre- and post -- prenatal and perinatal exposures, and how is that manifested in -- during the development. That was what I -- I didn't mean the standard DNT study either.

I'll let Dr. Horton comment, and then I'll come back to the next one.

DR. HORTON: Okay. In that context I think all three of us are in agreement in terms of, you know, at the current state of development of the data, the answer to the second question of are the neurotoxicity data more sensitive than the endocrine data. No, because the data are simply not good enough. They're, you know, they're clearly preliminary. That is absolutely clear. They're very preliminary.

What the data suggests is that there is a possible mode of action and a plausible reason to suspect that there is something going on that is consistent with the

suggested mode of action that has been discussed in context of the other question that is worth additional investigation.

DR. PORTIER: Dr. Reed.

DR. REED: Okay. So my second part of comment is this, that the endpoint for neuroendocrine aspect has not been clearly defined. I mean it's a mode of action, it's not an endpoint, and when it comes to endpoint for risk assessment, I'm not quite sure yet how that line is going to be drawn in terms of what is the manifestation of that and, you know, and so forth.

So given that the information right now is available, they don't look "more sensitive," but that, again, is just looking at the mode of action, not the dose response and not the result of the cascade of events that would come out at the very end in terms of the endpoint. I feel like it's premature for me to say that the neurological endpoints, including the neurodevelopmental and all the

other, you know, endpoints put together, is less sensitive.

I think that's probably a little bit different -- you know, less sensitive than the neuroendocrine type of endpoint, because if we're looking at either the same or similar mode of action or a different mode of action, we haven't even defined what that endpoint is. So we're saying there's not enough information, you know, from the neurotoxicity part of the database, it's not the same as saying because we don't have that, so it's not going to be more sensitive. Does that make sense?

DR. HORTON: I would say this is a case where there's a difference in the statement the data don't support versus there are no data. And in this case the statement is there are no data, not the data don't support. And so it's a need for more data.

DR. PORTIER: That was Dr. Horton. That's exactly what I was trying

to get. The point is I don't think they're answering your question about which one's better, and they're basically saying, we don't have enough data on the neurotoxin, neurobehavioral endpoints to be able to make that assessment. And Dr. Reed is leaving open the issue that a neurobehavioral endpoint may actually be the key health effect that they may be looking at.

DR. REED: Which we don't know at this point.

DR. PORTIER: Dr. Chambers.

DR. CHAMBERS: But I don't think these data sets are suggestive enough to say that you really need to launch a big effort to look for that at this point. I mean there's a little bit of stuff that's low dose in what we looked at, and it really is not showing a good dose response curve on the neurochemistry or the behavior or any of the endpoints that were looked at in stereology, any of that, to suggest that this is -- this has really got a

lot of plausibility in terms of displacing what you already know in terms of the most sensitive effect. If I`m getting the sense of what you're asking.

DR. PORTIER: I was going to make a suggestion that we leave this question open, take a break, come back right after lunch and continue, kind of -- because I feel like we're kind of going in a little bit of a circle here, no data, can't decide, not enough data to decide if we need to suggest even more research. So we're kind of caught in a loop here.

So let's break till 1:15. We'll reconvene at 1:15.

(Whereupon, the above-entitled matter went off the record at 12:04 p.m. and resumed at 1:16 p.m.)

A-F-T-E-R-N-O-O-N S-E-S-S-I-O-N

1:16 p.m.

DR. PORTIER: Before we get started, we need to kind of close off Question 1.5, and I just wanted to look over at Dr. LeBlanc and see if he'd had a brainstorm over lunch and whether he wanted to revisit it. If not, we'll close it off.

DR. LeBLANC: Well, I can say that in the hour I had during lunch, I thought about it definitively, I've worked out the mode of action, all the intricacies --

(Laughter.)

DR. LeBLANC -- but for the sake of staying on schedule, I won't speak.

DR. PORTIER: It'll be in the report. Okay. So I'll assume that Question 1.5 is closed now. But I just wanted to leave that open just in case.

And we still have before us Question 1.6. And my reason for leaving it open is still trying to get to this second

comment question, which is the more sensitive -- are the neurotoxicity endpoints examined are more sensitive than those elevated for neuroendocrine function. And I realize that there's lack of data and the two studies that we looked at, or the studies that we've looked at, have serious limitations.

But the question -- I think the question behind the question here from EPA is is this something they need to go and continue to get data on because it is likely to be more sensitive, or is it something they need to go get data on because it'll round out the issue, or is it not something they need to go get data on. I guess those are the three -- yes, Dr. Horton.

DR. HORTON: Okay. So you gave me a multiple-choice question. I think the answer to your question is B. And if B was that there -- no, it's not to just round it out, but it may -- getting more data -- no, it's actually all of the above, which wasn't

on your list.

The choices were, because the data -- the current data are insufficient to give us an answer to anything, if we have more data will it just round out our understanding of the mechanism of action of atrazine, will the provision of more data give us a better understanding of a more sensitive endpoint, and will it tell us whether there's a more sensitive endpoint.

And all of those things are true because it is possible and more relevant here to your regulatory and risk assessment, it's possible that if there are developmental effects, that those could be assessed at an earlier stage and recognized at an earlier point in an experiment and be more focused and with greater, shall we say, gain in an assay system, making for greater statistical power. If you're looking at -- particularly if you could put them into a neurobehavioral context where you can do multiple studies or multiple

measurements on an animal in a behavioral screen.

As have been done by many of the NIH cores that are doing behavioral screening cores for the neurogenomics screens, the mutagenesis screens where they're looking for point mutations in response to chemical mutagens, and they have batteries of behavioral endpoint, circadian rhythms endpoints, whole panels of neurobehavioral tests that they put the same animals through at different developmental stages, and we do this at Northwestern in our neurobehavioral core, of Rotarod tests and grip tests and the same animal will be put through 15 different types of tests to screen them for various anomalies.

And so you could put them through essentially a high-output screening test to look for a variety of things. And so if you can set up a screening program for that, you might be able to pick up a behavioral anomaly

on the same animal at a fairly efficient cost and with a great deal of power.

DR. PORTIER: So, but what's the expectation that that effect is going to occur at a dose, say, lower than an endocrine impact? And so that's one part of it. The other part of it is the difference between objective and subjective measurements. Right? And a lot of the neurobehavioral stuff is relatively subjective.

I know they're improving the methodology, but a lot of it, you know, you need many more animals -- they're not sacrificed, that's good -- many more animals to come up with that good definitive finding of a small behavioral change. At least the stuff that I've seen in the years we've been before here, whereas a lot of people like the objective measurements. Now you did mention some, you know, like genomic screens and stuff, those are more objective measures. Right? The kind of repeatability is pretty

high.

DR. HORTON: Well, I would say the kind of behavioral testing that I'm thinking of are things like circadian rhythms where you've got the animals on a running wheel, and I would counter by saying some of the weakness in the endocrine studies is that unless you're doing pulsatile bleeding for some of these endocrine measures, you're going to miss changes. And that is very labor intensive for what you're doing with the animals.

Many more of these behavioral tests are automated using motion sensor boxes, light detector, behavior -- oh, infrared boxes, there are a great deal more automation -- automated systems going into these to do high through-put screening of behaviors for these -- that have been developed for the neurogenomic studies because of the -- what I'm thinking of specifically are the ENU genomic screening systems. So --

DR. PORTIER: I think this is

good.

DR. HORTON -- in terms of looking at low doses, I think many -- mainly it's because many of the developmental effects may be sensitive to lower doses than adult response, and you just don't know what you're going to pick up when you start looking at the low doses because those experiments haven't been done yet.

DR. PORTIER: I have a follow-up question for the Agency. If atrazine were newly registered today, and hadn't been around for 50 years, would a neurodevelopmental or a neurotox battery be required of the registrant as part of the standard package? I mean would we have had something like that in the standard packet?

DR. MENDEZ: Elizabeth Mendez, EPA.

Within the last two years the new CFR, the new Code of Federal Regulations, we have a new set of guidelines, and under those

conditions an acute neurotox would have been required. A DNT is still not, and a subchronic as well. So an acute adult neurotox and a subchronic neurotox would have been required. A DNT is still a tier two test, and DNT is the developmental neurotox.

DR. PORTIER: Dr. Chambers.

DR. CHAMBERS: There are plenty of objective measures in behavioral tests, so that's -- it's not all fuzzy subjective stuff. With all due respect, I'm going to disagree with you. I know that resources and time and everything is really limited, and I don't really see anything in the data sets that we were provided with that suggest that doing some extensive developmental neurotoxicity, not DNT testing, but any kind of extensive developmental neurotoxicity testing would really be a wise use of time and resources right now considering the much more extensive database you have already presented to us on the neuroendocrine effects.

So I don't think there's really anything suggestive in those particular papers that were given to us that suggest a very extensive array of neurotoxicity testing is done. I'm just a little bit afraid that it might be nothing more than a fishing expedition and that you would not find anything at lower doses that would, again, displace the neuroendocrine effects as the most sensitive effect right now.

DR. PORTIER: And so there's the other issue though is the developmental versus adult. All right. I mean part of what I hear Dr. Horton saying is developmental neurobehavioral and so the question is whether the endocrine data and endpoints are sufficient at the developmental stage. I mean, you know what I'm kind of saying? Because most of the studies we've looked at are adult animal studies. Right?

DR. CHAMBERS: Well, a couple of these -- this is Jan Chambers again -- a

couple of these were early treatments, but they were not well-powered for the statisticians, they were not well-conducted, you know, not standard types of tests and all. So, again, the data are not good in terms of dose responses and so forth. So, again, you know, in my mind, I didn't see anything that really suggested that we should rush right out and expand these types of studies.

DR. PORTIER: I guess it's my understanding there'll be more data coming on some of that as well. Right? Some of the --

DR. MENDEZ: That is correct. We have more data coming in within this week.

DR. PORTIER: Dr. Horton.

DR. HORTON: Yes, well, one of the areas where we disagree is over the quality of the data. And I guess the metaphor here is whether you are feeling the elephant in the room or not. And while each individual study is not of high quality, when you look at each of these individual studies, I'm feeling an

elephant in the room that suggests that there may be something going on in terms of the effect on the nervous system.

And each of the nervous -- the neural systems that is impacted or suggested to have -- to be influenced by atrazine in these systems, the dopamine system, the serotonin system, the -- what do we have in here, the somatostatin system -- not so much the somatostatin because that is influencing growth hormone, but it feeds back in there someplace, but primarily serotonin and the dopamine systems. And as you look at how widespread those areas are, when you think of their impact on cognition, when you think on their impact on affect and attention.

You need to consider not just what impact they may have on reproductive function, on the HPA axis, you need to consider what impact they have on mental disorders, schizophrenia, affective disorders, executive function disorders. There's a wide range of

issues.

You need to consider the relationship between estrogens and their inter-relationship with insulin signaling and metabolic disorders. We're walking into potentially a broad range of effects. There are insulin receptors in the brain. Anybody who had physiology or biology 20 years ago probably thinks of glucose and insulin, insulin not having an effect in the brain, and glucose uptake being constant in the brain.

Well, that's true, but there are insulin receptors in the brain, and those insulin receptors are interacting with estrogens, and they're influencing the signaling in the brain. So as I feel this elephant in the room, and I feel the dopamine system potentially being influenced, I think we need to look at this.

And when I look at graphs that, yes, they have small sample sizes, and the data were collected by stereology, which may

or may not have wide acceptance, but the stereology is a relatively new and highly sophisticated method for collecting data. It requires exceptionally expensive confocal microscopes, which are on the order of tens of thousands of dollars. It requires considerable training in using that microscope to analyze the micrographs. These are not simple techniques.

Or I consider that journal editors consistently require people to cut down the amount of images that they put into papers. I'm not disillusioned or concerned when there's not a micrograph in a paper. But when I do see that an author has given me not just the bar graph and the standard error of the mean, but they have given me the scattergram that includes every individual point for the animal so that I can compare the distributions for myself and look at the overlap and see what those data look like, and know what that elephant in the room feels like.

I'm willing to think that we do have an elephant in the room, and I think we need to accept that we have an elephant in the room. And I think we need to evaluate the effects of atrazine on the brain in multiple situations. Thank you.

DR. PORTIER: I think what I'm going to recommend to Dr. Horton and Dr. Chambers and Dr. Reed is when we put this together, we don't necessarily need consensus, but we need all of these arguments kind of filled out. And I think you've done that. So I'm not worried about coming to consensus on this one. I pushed, but it's clear that we're not going to get the panel to agree on this.

And I get the feeling Dr. Horton's on one side, Dr. Chambers is kind of on the other, and Dr. Reed's kind of in the middle. So we'll be looking at Dr. Reed to kind of build that transition between the two.

Any additional comments on this question?

DR. REED: I was asked if I'm ambivalent. No, I'm not ambivalent for being in the middle. Yes, but I think, you know, my comments were --

DR. PORTIER: No, I think of you more as cautious and unwilling to give up on these potential health effects --

DR. REED: Right. Right.

DR. PORTIER -- as a potential point of departure.

DR. REED: Right. And the concern was really just based on the mode of action, as we've been talking about these two or three days, and in addition there might be, you know, other sidebar on the mode of action which is not completely deciphered, but I am concerned about developmental type of effect with in utero exposure, perinatal exposure, and the sensitivity issue in that I think it needs to be addressed.

DR. PORTIER: And it will be.

Yes, Dr. Lowit.

DR. LOWIT: I would, unless Dr. Cooper has something -- or Dr. Mendez had something to add, I would, I guess, follow your line of thinking to have the report represent the variety of opinions, and I guess putting a happy face on coming back two or three more times.

We do have the September meeting, and I'm not aware that anything new will come up on this issue, but we may be able to, when we have a newer set of points of departure and a fuller evaluation of the life stage effects, this may become -- there'll be more clarity with a more full analysis around the relationship of all these, and we can make a more robust description, I think.

DR. PORTIER: Okay. I think we'll close out on this question. That ends Question 1.6, and we're going to move on to Question 1.7.

DR. COOPER: I'll read this question. I'll keep the ball moving anyway.

Charge Question 1.7, based on a review of two studies of the potential effects of atrazine on the prostate, Section 3.3.2.2 of the draft issue paper, the Agency has concluded that the results of the Rayner, et al., study 2007 -- the Rayner, et al., 2007 study of pregnant rats treated during gestation support previous observations, Stoker, et al., 1999, that atrazine treatment to the rat dam either perinatally or early postnatally can increase prostate weights due to an increase in inflammatory infiltrate in the male offspring.

This effect on the offspring was shown to be due to a suppression of prolactin in the atrazine exposed dams during lactation -- which I might add is in contrast to the Stoker paper where it was done postnatally -- and is consistent with the mode of action of atrazine on neuroendocrine function.

The Agency also preliminarily

concluded that further research is needed to provide more convincing evidence that atrazine-mediated suppression of prostate cancer in the probasin/stroke SV40T antigen transgenic rat, androgen-dependent prostate cancer rodent model, may possibly be due to caloric restriction rather than endocrine-related as postulated by Kandori, et al., 2005.

Please comment on these preliminary conclusion regarding the recent prostate findings. Please comment on the extent to which available data support the proposed mode of action for prostatitis, and the appropriateness of the rodent model in the context of human health.

DR. PORTIER: Dr. Williams.

DR. WILLIAMS: So this question gives us some questions about studies that we really haven't talked about at all yet, so I'm just going to briefly review the two studies in question and then answer these.

So the Stoker, et al., `99 paper demonstrated that postpartum administration on day 1 to 4 postpartum of doses between 6 and 50 milligrams per kilogram per day of atrazine to lactating rat dams dramatically, or in the higher doses completely, inhibited suckling-induced prolactin release in the dams, and that it also caused an increased incidence of lateral prostate inflammation in the male offspring at four months of age.

They observed the same effect after treating dams with bromocryptine, which is a dopamine agonist that inhibits prolactin release. And this effect was reversed by the administration of prolactin to the atrazine-treated dams. So they basically saw an effect and they also reversed the effect, and so it was a really very nice study in that way.

The proposed mechanism to explain these results was that prolactin is important for neonatal brain development, and lack of neonatal prolactin exposure from getting

prolactin in the milk leads to a failure of dopaminergic system development -- again, we're talking about this sort of neonatal effect on the brain here -- and eventual hyperprolactinemia in the adult male.

The study didn't actually demonstrate hyperprolactinemia, this was based on previous work where that had been shown, that hyperprolactinemia can occur at about the one month old male after this kind of an exposure. And the prolactin levels were only measured at four months of age, and at this time there was no further hyperprolactinemia, so they were different from controls at that point. But the proposed mechanism was that there was this period of hyperprolactinemia that led to prostatitis.

So the newer studies, Rayner, et al., in 2007, examined whether treating rat dams with 100 milligrams per kilogram per day of atrazine during late gestation, in day 15 to 19, had adverse effects on prostate

development of the male offspring at either four or seven months of age. And in this case they actually used a cross-fostering design to distinguish between direct effects of prenatal exposure of the atrazine that's given during gestation, or was if effects of the prenatal exposure to atrazine on the mom with milk, and so the cross-fostering design was used. And so they basically took pups from controls and had them suckling from the previously atrazine-treated animals, and then they also some atrazine -- animals from atrazine-treated dams that were moved over to control animals. So it was a really nice cross-fostering study in that way.

So this study demonstrated a lot of similar things, that they -- suckling from an atrazine-exposed dam resulted in an increased incidence of prostatitis, whether or not the male was exposed prenatally to atrazine. So it looks like it's a postnatal effect that is the sensitive time, not

prenatal. And that was consistent with the Stoker findings where they only treated postnatally and got the effect. Here if the pup didn't suckle from the atrazine-treated mom, there was no effect. So if they went over to the controls, they did not have the prostatitis.

So regarding the proposed mode of action, the Stoker results demonstrate a clear connection between diminished maternal prolactin levels and prostatitis in the male offspring. However, the proposal that the prostatitis was due to hyperprolactinemia in the male offspring wasn't tested in these studies, as I mentioned. The prolactin levels were only measured at termination of the experiments at 120 days of age and were no different from controls at that time. They clearly could have been elevated earlier on, and that just wasn't measured here.

One way to test that question would be to follow prolactin levels and

document that indeed they are elevated during some time of the peripubertal period, or potentially blocking hyperprolactinemia at that time by administering bromocryptine to see if that's actually the mechanism to explain the prostatitis because there's also the possibility that there were atrazine metabolites or something in the milk that the pups were exposed to, and that these metabolites might have affected the brains directly or done something else that led to the prostatitis later.

The reason that this matters is because it would be important to know if it's something in the milk that's a problem or something not in the milk that's the problem if we're going to think about human health effects later. For example, if the problem was related to diminished prolactin levels in the milk, then maybe we ought to be worrying about the effects on human health of failure to breast feed, which is true of about 30

percent of women in the United States.

On the other hand, if it's something due to having atrazine metabolites or something else in the breast milk, we need to worry about the 70 percent of women who are breast feeding. And so, kind of getting back at is it really important to figure out the mechanism in this particular case, it may be because it'll affect how we're going to think about the exposures to the pups.

So now I'd like to move on to the Kandori findings where the Kandori paper, looking at prostate cancer and the probasin/SV40T antigen-transgenic rat, the paper basically supported the idea that atrazine-mediated suppression of prostate cancer in this model was due solely to caloric restriction and not -- and was not an endocrine-related outcome.

And I think that's an important question given that there is a lot of literature where, at least in the high-dose

atrazine studies, there is clearly a caloric restriction effect, where given high enough doses, animals will lose weight and maybe the weigh loss could end up explaining findings later.

Unfortunately, there's a lot of problems with this particular study, and so I think at the end the Agency's conclusion that it's leading you to uncertain findings is generous at best. And there were four problems that I'll outline. One is that the atrazine dose that was administered is very uncertain in these studies. They were using dietary administration, they had two rats per cage, they don't really know what each rat got. And the diets, when they went back and measured what the diets actually had in them, they didn't have in them what they thought they had put in them in the first place, and so the intake couldn't really be calculated.

The testosterone levels that they saw in these rats were not different, even

though they had similar doses, where in other studies testosterone levels were different in the atrazine-treated animals. But the atrazine dosing might have been so variable that you couldn't find results. The testosterone levels had standard error bars that were basically as large as the bars themselves. And so it was just a lot of variability, it was so hard to make a conclusion.

The caloric restriction group themselves had highly variable testosterone measurements, and so was it caloric restriction and other issues with that, or was it something to do with the testosterone. It's really not clear. And then at the end of the day this was a model where 100 percent of the animals have adenocarcinoma or other -- or intraepithelial neoplasia in the prostates at 15 weeks of age. These were looked at 13 weeks of age, and indeed pretty much 100 percent of the animals had the problem.

What they showed was that with either the atrazine or the dietary restriction and caloric intake there was a very, very slight decreased if you measure the number of epithelial cells as a ratio of epithelial cells to total prostate size. And it was a very small change and it was not a change in overall levels of cancer at all. And given that they only did these measurements on literally two slices through the prostate and they didn't do serial sections that encompass the entire prostate, the findings were so minimal that I'm not real enthusiastic that another person doing the exact same study would come up with the same results.

So interesting idea, potentially could complicate studies if you have to think about caloric restriction, but I'm not sure that this particular study should be given really any weight in terms of what the EPA decides to do.

So regarding the appropriateness

of a rodent-model in the context of human health, there really is a consistent finding in both rats and humans of an association between prostate inflammation and proliferative inflammatory atrophy lesions, prostate hypertrophy, intraepithelial neoplasia, and cancer. And there's enough findings in human literature in that regard that there's actually been clinical trials looking at non-steroidal anti-inflammatory agents for the attenuation or treatment of prostate cancer.

So the link is really strong in the human literature, and certainly it seems to be just as strong where prostatitis is seen in association with prostate neoplasias in rats. So there's a lot of similarities to the two systems, and I think that that says that it's a reasonable model.

In terms of thinking about prolactin exposure -- sorry, loss of prolactin exposure and effects through lactation, I

think we need to know a lot more about the mechanism before you can say that's a reasonable model.

DR. PORTIER: Thank you.

Dr. Delclos?

DR. DELCLOS: I'm in agreement with those comments, and I really have nothing further to add.

DR. PORTIER: Dr. Horton.

DR. HORTON: I'm in agreement, and I have nothing to add.

DR. PORTIER: Somebody else, please? Dr. Heeringa agrees.

Dr. Cooper.

DR. COOPER: Just to follow up one thing. There's, again, the time line's a blur, but I don't believe this was post-2003. We had a graduate student working in my lab who essentially followed through on that. The hypothesis that was being examined, it was based on Crowley's work earlier on, was that if you inhibit prolactin, tubular and

fundibular dopaminergic neurons won't mature. And that was based on a number of different studies where they showed if they're not there, they don't get momma's prolactin.

And so what Chris Langdale did, and this is his thesis work, which is through NC State tox department, he showed that the -- he looked at the dopaminergic neurons' developing, and indeed there was a delay. Where the change occurred most dramatically in terms of prolactin regulation was between PND27 and 35, peripubertal change. And it occurred only at that time, and that matched very well back with some of those -- Judy Ramelay's work looking at maturation of prolactin regulation.

So there was some mechanistic -- again, these are piecemeal things, but I think they're supportive of that, or point in the direction that there may be impairment in that titis system based on that kind of exposure. And whether that is an impact -- these neurons

grow -- they mature up to birth and then they require the mother's prolactin, so whether the preterm treatment is going to influence her ability to release prolactin and then that's something that I don't believe has been followed through,

So, no, we didn't measure both before and after birth, but mechanistically anyway it's -- the data that Chris Langdale had was consistent with that dopaminergic hypothesis. I think it speaks somewhat to this do we see developmental effects. I believe we've looked for developmental effects that maybe looking under the light, but they're there.

DR. PORTIER: Dr. Horton.

DR. HORTON: Dr. Horton. I was just going to comment, but Dr. Cooper said it for me, is that what he just said about the prolactin changes in these developing males points to a developmental effect of the atrazine on the dopamine neurotransmitter

system, which is relevant to Question 1.6.

DR. PORTIER: I don't see any additional comments, and I can't really summarize her summary, which was pretty good. So we'll make sure that's in the report.

Okay. Gosh, I was thinking we might not get to the hydrology today. So take your time reading Question 1.8.

DR. LUEBKE: Okay. On to Question Number 1.8. Based on data obtained in studies to assess the effects of atrazine on the immune system following developmental and adult exposure, as well as in vitro mechanistic studies, EPA concluded that atrazine has the potential to affect the immune system. However, the Agency has also concluded that underlying mechanisms of atrazine-mediated immunotoxicity -- you'd think I could say that word by now -- and its relevance to potential adverse health effects in humans are still not thoroughly understood. It is well established that products of the

endocrine system modify immune function. However, adult rodent studies have not determine whether immune system effects are caused by direct effects of atrazine and/or its metabolites, or whether they are the result of modulated endocrine hormone production, which in turn affects immune cells.

Two published studies indicating that exposure to atrazine during immune system development may result in altered immune functions in offspring. Although no conclusions were drawn on the potentially -- on the potential adversity of these effects because immunosuppression was observed in one study and immunoenhancement was observed in the other.

Please comment on the potential explanations for the disparate findings reporter by Rooney, et al., and Rowe, et al. Many immunotoxicologists consider immunomodulation -- i.e., suppression or

enhancement of immune function -- as a potentially adverse alteration of homeostasis because both have been associated with disease states. Thus, while the results of Rooney, et al., and Rowe, et al., may appear to be contradictory, together the data indicate unintended immunomodulation at approximately the same dose in two species.

Please comment on the effects of gestational atrazine exposure as immunomodulation when describing the immunotoxicological outcome of developmental immunotox study -- immunotoxicity studies.

Appendix -- shall I keep going?

Okay. Appendix B of the draft issue papers describes experiments conducted by EPA scientists on the potential developmental immunotoxicity of atrazine. The results for the experiments provide conflicting results. Additional studies did not provide a suitable explanation of the differences. The Agency believes both sets of

data are of high quality. However, in the context of hazard assessment, such differences are difficult to interpret. Please comment on the information contained in Appendix B and provide suggestions for interpreting such data as part of the atrazine re-evaluation.

Oh. Oh, okay. Here we go. We're having so much fun.

The available data -- this is a long question too -- the available data do not indicate that atrazine-induced immunotoxicity is a more sensitive endpoint than the atrazine-induced effects on neuroendocrine function, e.g. attenuation of LH surge and estrous cycle disruption which form the basis for the current chronic RfD in female Sprague-Dawley rats. Please comment on the degree to which these preliminary conclusions are supported by the available data.

DR. PORTIER: Dr. Holladay.

DR. HOLLADAY: The text for that question was longer than any others. We ought

to petition for a higher per diem or something for that.

Ken, we talked as a group about addressing each sub-question, I think there were four in here, one at a time and maybe it being more functional if we did it that way.

The first of those is the Appendix B question, which Dr. Luebke covered very well the day before yesterday, I think it was. So I'll just review that very briefly. These are the Rooney, et al., studies where the hypothesis that atrazine may cause developmental immunotoxicity through disruption on prolactin or thyroid hormones was tested in Sprague-Dawley rats using a dose of 35 milligrams per kilogram per day by oral gavage. This is from gestation day 10 through postnatal day 23, I think it was. And they chose that dose to be just above the NOEL for delayed onset of puberty in female offspring.

They had separate groups exposed to bromocryptine and to propylthiouracil to

produce hypoprolactinemia and hypothyroidism respectively. Neither of those two groups caused immunosuppression in the offspring. The experiments were replicated twice. The offspring did show immunosuppression at about eight to nine or ten weeks of age. The atrazine decreased the primary antibody response to sheep red blood cells and decreased the delayed type hypersensitivity response in adult male, but not female, offspring.

I think these tests were probably chosen or selected in part from the National Toxicology Programs Risk Assessment for Immunotoxicity and positive data would predict a positive response also in challenge assays if we were to proceed that way. So it's a good testing, good experimental design. I read the papers, I put in the graphs. The suppression looked real and I think it was at about 56 to 59 percent of the control level.

You can ask questions were p-

values -- was chance playing a role in this one time out of 20, will p-value let you down just because of the level we set? I don't think that's the case, but with one chance out of 20, and then in a replicate experiment multiplying that it shouldn't occur more than one time out of 400 twice in a row on you for one assay, and then we've got the DTH as a second assay that did the same thing, the experimental design looked good, the data looked real. The authors concluded that developmental exposure to atrazine caused a gender-specific suppression of immune function in the adults that didn't appear to be through prolactin or thyroid hormone, the mechanism.

So that's an overview for the studies. The difficulty that we were asked to consider came from the further repeat of these studies when the lab was moved from old EPA facility to the new one across the lake from NIEHS, and then didn't replicate for the investigators in spite of same protocol, same

lab people doing the assays. So 35 milligrams per kilogram per day was the high dose selected in the repeat study, the same as the first study. Lower doses of 17-1/2 and 3-1/2 milligrams per kilogram per day were used. This was an attempt to establish a dose response for the effect observed. End data were negative.

Second experiments used Sprague-Dawley, Long-Evans, and Wistar rats to look for a strain effect. Strain differences data were, again, negative. The assays were conducted only in males given the results in the first set of experiments. So the investigators concluded that some unknown environmental factor was probably behind the difference in response. They have a high level of confidence in the technical soundness of the published studies.

From my review of those, I see no reason to think otherwise and agree with this unknown environmental factor. I think it was

Dr. Akana who suggested that vibration in a new facility or something like that could play a role. I know last week someone from the lab came to my office with those conical centrifuge test tubes we hold our cells in, the orange-topped ones, and said these were from the last two batches we'd gotten.

Opened one and he said, smell it, and I smelled it. I said, I don't really smell anything. He said, Okay. Opened the next one, he said, smell this one. It smelled like a plastics factory or a new car or a carpet. And he said, I've never smelled that in these before, but I'm afraid that could skew our data, whatever these things are emitting could be harmful to ourselves, and he was concerned enough he called the vendor, who said, Yes, we've outsourced our production to Mexico.

Don't know what the difference was, but sometimes there are little things that do this to us that cause differences like this. Unknown environmental factor was a good

disclaimer, I think, for that, and we get caught in it now and then. So my conclusion is there's no reason to doubt the results of either study in this case, and I think the original study was conducted appropriately and the results should be accepted as real under the conditions of that study.

DR. PORTIER: Okay. Done with the first part.

DR. REGAL: Right. So with regard to the Rooney study --

DR. PORTIER: Dr. Regal.

DR. REGAL: With regard to the Rooney study, everything looks very sound in terms of methods, techniques, design, and, you know, clearly a critical parameter has not yet been defined to explain why it can't be replicated in another status.

DR. PORTIER: Dr. Bucher.

DR. BUCHER: So I'm not an immunotoxicologist, but I do have the pleasure of having attracted Andy Rooney to our program

from EPA, so I talked to him about these studies, and I just wanted to have a placeholder in case the discussion went off in an area where he would have disagreed with some of those conclusions, but he agreed with everything that has been said about those two studies -- and that they were, in fact, excellent studies and just for some reason didn't repeat. So I agree with those comments.

DR. HOLLADAY: All right. So Holladay back. Now the second question we're asked to address was the dichotomy, if you will, between the Rooney, et al., results 2003 and Rowe, et al., 2006. The difference between these studies on one reported postnatal immune suppression, one reported enhanced immune responses. These are in the adult offspring after the developmental exposure.

I think the most important thing to consider between these two studies is that

there were many differences between experimental design. The Luebke lab used the Sprague-Dawley rat and out-bred animal; the Rowe, et al., studies, which is John Barnett's lab in West Virginia, another internationally recognized laboratory, both the laboratories are of that stature, used Balb/c mice. So we've got different experimental animals. The Balb/c mouse is a T helper 2 skewed mouse which is skewed towards enhanced antibody production. So that's one difference that's important.

Exposure route is also important. The Sprague-Dawley rat study is oral gavage, which provides a bolus or spike exposure of the atrazine to the pregnant animals. The Barnett lab used implants that provided a lower level time release. The doses were comparable. The Rowe, et al., studies calculated their dose varied from about 35 to 23 milligrams per kilogram per day atrazine. This is in the mice, again, from gestation day

10 to 12, so this is a shorter exposure as well.

So both studies were well designed, in my mind, robust, used statistical power analyses to calculate the needed N. The Barnett study used N of 11 group size, which is larger than I've tended to use in a study like this.

So the results are seemingly different, but when we consider the endpoints evaluated, when we consider the models, I'm not sure they are -- well, it's not worrisome to me that they're different. I commented the other day to that we're seeing more results of this sort, even within strains where we see both immune suppression and immune enhancement coexisting in the same animals.

It was kind of a paradox when we first realized this was happening, but it does. DTH, delayed-type hypersensitivity, or T cell response might be depressed, while other T cell activities are skewed or

enhanced.

So we've got genetically different animals and one is showing a depressed T cell response, the DTH response in the rat study, and others showing an enhanced response in the mouse study, the MLR or proliferation, T cell proliferation outcome, and the cytotoxic T lymphocyte activity and other T cell activity, as well as enhanced antibody production. So different results but very different models, results not inconsistent with literature in other mouse models with different chemicals.

So as the Charge Questions indicated, there are seemingly different results, but in my opinion these should, at this time, be both considered as observations that represent true outcomes for the models and experimental systems used.

DR. REGAL: Jean Regal. I definitely agree, I just have a couple of differences to add for the record in terms of the end result. They were looking at eight

week old rats and 12 week old Balb/c mice as to their timing, and I have no reason to think that those are different in terms of being post -- past sexual maturity. They used two different vehicles, time-release pellets versus methylcellulose gavage. Again, no reason to believe there would be a difference.

One question I would have for people who think about atrazine metabolism is would the metabolism differ depending on the route of administration, i.e. one gavage versus sub-Q administration? Could some of the metabolites be, you know, causing an immunoenhancement versus some an immunosuppression? So I guess I'll be looking -- and any comments people have on that would be appreciated.

And the only other -- one other thing in terms of the rat study, there was a very small decrease in weight of the seven day old rats, and with the Balb/c mice there was no weights at birth so the very small chance

that any of this might be -- have been done to intrauterine growth restriction rather than an effect of atrazine.

DR. BUCHER: So I have nothing really to add. I do agree that while it would have been nice if these changes would have gone in the same direction, it doesn't necessarily detract from the fact that they are real effects, so.

DR. PORTIER: Well, why don't we open up and see if there's any metabolism comments.

Dr. Hayton.

DR. HAYTON: Yes, I seem to remember the McMullin 2007 paper which was a physiologically-based PK modeling study, and it's far from definitive, but the kinetics suggested that there was a very substantial pre-systemic elimination for the oral route. And so that I think more than -- something more than 60 percent of the atrazine was converted to metabolites pre-systemically.

Well, that clearly wouldn't happen following subcutaneous administration. So there definitely could be route of administration differences in metabolite exposure.

DR. GREENWOOD: I also came to that sort of conclusion because for -- in rodent studies, the profile for atrazine itself is really a text book case of a picture. It's consistent with a very low rate of absorption and a rapid rate of elimination and the production particularly of the diamino chlorotriazine, which is sort of the end product down that route.

The production of it was so rapid that really I can't see any other explanation, other than it was by the enterocytes. And this has been seen for drugs and other things that this happens.

And you get similar rates of absorption in humans. You can predict human absorption from the rat pharmacokinetics, but the

bioavailability by the two is totally different. So the rate of absorption is similar and highly correlated in humans and rats, but bioavailability by the oral route is different. And the difference comes because of this metabolism differential, metabolism in the gut wall.

And it's something that's known, really going back a long way where people used C14-labeled phenols which were administered orally and none of them reached the liver intact, so where people were looking in the hepatic portal vein. So it wouldn't surprise me at all to different species and totally different routes of application if you wouldn't get totally different patterns of detoxification products.

But what I would guess is, looking at the -- I don't know whether the others agree -- but looking at the profiles, it just seem that this diamino chlorotriazine seems to be the major detoxification end product. I

mean it was something like a hundred-fold different -- higher than any of the other compounds after that 24 hours.

DR. PORTIER: Dr. Horton.

DR. HORTON: Yes. One comment. Even though we're considering the animals held in a laboratory environment under constant photoperiods and that laboratory mice are not photoperiodic, some strains of laboratory rats still show a photoperiodic response, specifically the Fischer 344. And in other species, specifically the dwarf hamsters, Phodopus sungorus, they have been shown to show photoperiodic dependent changes in their immune response, and specifically the cellular versus the humoral immune responses differ on a seasonal basis.

So I'm wondering whether in looking at these data, both the rat strains, you know, Fischers versus Sprague-Dawley and also the photoperiods might need to be taken under consideration.

DR. SCHLENK: Yes, just to follow up on the metabolism issues. I know -- I mean as I mentioned earlier, there's some really good evidence to suggest that it's a 3A4 substrate, which is actually expressed in fairly high concentrations in the gut, so that would argue for enteral metabolism immediately without a systemic absorption at that point.

So there is that, although recombinant proteins, I think the high -- the more efficient catalytic pathways 1A2, which would be more liver-based, but again, I think arguing more for a -- if you do have 3A4 under high KM, high sort of concentration-based doses, then you probably would get the gut metabolism preceding the hepatic.

DR. PORTIER: Dr. Luebke, you had wanted to make a comment?

DR. LUEBKE: Okay. Well, I just wanted to respond to a couple of things. So photoperiodicity, a fellow that I know who's done thousands and thousands of immunotox

studies took all of his mouse data from over the years, just the control data, and he graphed the antibody response and the delay type hypersensitivity response, and what you could see was sort of a nadir in the spring, and as winter came on, it went up.

So mice are, indeed, photoperiodic in at least -- you know, over the year. Over the year. I shouldn't say photoperiodic. Okay. So, yes, so there is annual cyclicity in all that that can't be explained by seeing sunlight because these things never see sunlight unless they've just come out of the truck.

The other thing that I would like to mention is the issue of metabolism, and I too was concerned with this and I thought that there may be some questions about this because of the different, very different routes of exposure. And so I talked to Mike DeVito, who used to be EPA and is now at NIESH and is something of an expert on pharmacokinetics,

and he assured me that there would be massive differences in the amount of atrazine, per se, that was likely to seen by these animals that got the subcutaneous implant, because it's not going through the gut, there is no first pass through the liver, and if -- assuming that it partitions into the blood and goes all over the place, then, yes, there's going to be a big difference.

But, now, Ralph and Tammy may have to help me out of a quagmire if I start to dig one here, but it's my understanding that if you look at the parent and the metabolites, that there are differences in the metabolites in their action on the HPG axis and maybe the HPA axis. If we assume that the effects that we saw here are somehow related to that, and not a direct effect of atrazine itself or it's metabolites on the cells themselves, then it would not be beyond the realm of possibility, I think, that if you consider that what we might see there, because of the differences in

dosing, might be something that's quantitative and not qualitative, because if atrazine and its metabolites are going to effect HPA to HPG and then drive these different effects that we see, then I don't think that they would necessarily go in opposite directions.

And I had hoped that I could come up with some different, you know, explanations because it would be real nice to say, well, yes, it's a metabolite thing and that's why we got enhancement in one and suppression in the other. But I still come back to the original idea here that what we've got were unexpected changes in function, neither of them necessarily good.

DR. PORTIER: Dr. Greenwood.

DR. GREENWOOD: So I think that sort of differences that you're looking at would be larger in terms of exposure probably to atrazine itself because it would much higher, as I think has been said by the implant. You're still going to get DSET

eventually formed as you go round the circulation system, it comes through every tissue eventually, and that's, you know, part of the reason we look at the area under the plasma curve as the opportunity factor for reaching any particular tissue, whether it's a target tissue or an non-target tissue.

But I think there are other factors which can come into play as well because if you have a high dose particularly, then you start -- I think someone else alluded to this yesterday -- you start to get differences maybe in -- changes in the glutathione pool, and as that's involved in metabolism of all sorts of other things, then you could start to produce different results.

And so I mean I haven't -- I would need to -- really to see the sort of concentrations people are getting from the implants over the period of time compared with the -- what you get by oral gavage. But oral gavage is certainly something which is often

very difficult to interpret this sort of absorption because the corn oil or the methylcellulose forms part of the system, it becomes part of the metabolic compartment, and it changes with time.

So you get, you know, rapid absorption initially, through for instance the stomach wall, of what's free, and then it takes time for the other stuff, particularly with atrazine, I think, with its properties it seems to stick to things, it takes longer to disperse from the bolus. And so you get a longer -- then a longer term lower level. And if you've got metabolism as it's going through the gut wall, then very little of the atrazine itself is actually a component at very, very low levels, and you won't get that if you've got an implant. You'll have a more consistent and prolonged level of atrazine.

DR. PORTIER: Dr. Reed.

DR. REED: Any reason to think that male/female differences seen in any of

the studies could be explained by metabolism?

DR. GREENWOOD: Sorry. I haven't seen that, anyway I've not seen any evidence for such a difference.

DR. SCHLENK: This is Dan Schlenk. Yes, as far as I know, I don't think there are any male -- gender differences in 3A4 in rats. I know there are the two -- family two enzymes, but not necessarily the threes which would be more appropriate for this, but.

DR. PORTIER: Okay. Back to the question. Dr. Holladay.

DR. HOLLADAY: All right. Let's continue. The third question was, Please comment on characterizing the effects of gestational atrazine exposure as immunomodulation. This is rather than as describing them as immunotoxic. And I think, after what we've just said, that's the appropriate descriptor, immunomodulating allowing for both suppression and enhancement of immune function after a developmental

exposure.

So I agree that's the correct descriptor to use and agree with the Rowe, et al., conclusions in their studies that this potentiation of an immune response is an important outcome, may potentiate diseases such as auto-immune or hypersensitive diseases, suggest the need to evaluate the ability of developmental atrazine to enhance postnatal immune responses in mouse models that are genetically prone to express such responses. So I have to wonder what would happen in a mouse model that was genetically predisposed to auto-immunity for instance. That'd be an interesting question to have answered.

So, yes, immune modulation is the correct descriptor for these data.

DR. PORTIER: Dr. Regal.

DR. REGAL: Yes, I agree, with the immune system, whether you get too much of it or too little of it, it can be an adverse

event.

DR. BUCHER: This is John Bucher. I also agree with that.

DR. PORTIER: Any additional comments?

(No response.)

DR. PORTIER: It's a long question, but you took it apart and answered it. That's great.

Any comments from EPA? Oh, there's one more. I'm sorry. It was a long question. I only thought he had broken it into three, and it's four.

DR. HOLLADAY: Maybe the most important part. Do the available data support immunotoxic, or immune system endpoints, or immune modulation as more sensitive than the atrazine-induced effects on neuroendocrine function, the attenuation of the LH surge or estrous cycle disruption. Please comment on the degree to which these conclusions are supported by the available data.

And I wrote as my opinion, I agree that the available data indicate that neuroendocrine reproductive effects are more sensitive than immunotoxic or immune modulation effects thus far detected. I support the attenuation of the LH surge with NOEL of 1 point milligrams per kilogram per day as the appropriate point of departure from the available data.

I just stress that it should be considered the existing developmental immunotoxicity database is quite limited and further studies would be interesting. I think neurodevelopment -- we could argue for more development of immune system, we could certain argue for more studies. But existing database, I support the current point of departure that we're using.

DR. REGAL: And I guess I do to the same extent that -- except with the caveat that with the developmental immunotox studies, we really only looked at one dose, and

nobody's gone lower than 35 milligrams per kilogram per day for 14 days, I think, or for 20 -- however many days they did it. And so because of the disparate results there, and because of -- to me I always try to remember that in immunology stuff, you're going to see bell-shaped dose response curves where you have nothing with a lot, and nothing with a little, but you get stuff in between. And so we might not have hit those in between doses. I think lower doses should be assessed developmental immunotox-wise.

DR. BUCHER: And I agree with those comments. I think that there was an attempt to do a dose response study, but it was not successful. But I think that that certainly would strengthen the database, and it's nice to see developmental studies, and I'd certainly like to see them as well for the neurobehavorial effects.

DR. PORTIER: I almost expect Dr. Horton to jump in here. Anyone else on

metabolism? No? Yes, Dr. Akana.

DR. AKANA: I'd just like an opinion from the metabolism crew on what would -- what happens with atrazine when it's mixed in the diet compared to say the gavage delivery. Would you expect the same concerns about the factions of metabolites of atrazine?

DR. PORTIER: Dr. Greenwood.

DR. GREENWOOD: Yes, I think that if you've got a more continuous intake then you're still going to get a very low absorption from the diet. I think that the atrazine, because it's very insoluble, it's got to move out of the diet into the water before it can cross -- there are several barriers, there's the mucous layer and there's the understood boundary layer, or the boundary layer of water, not understood layer, the boundary layer of water, and before it reaches the epithelium.

And I think the key stage in all of this, if you look at the papers of people

like Mike Abraham and so on, seems to be the dissolution. And I guess actually the kinetics of movement out of food will not be too dissimilar from the kinetics of movement out of the material that's used on oral gavage.

But having said that, if it's in the diet of course then it tends to be taken over longer periods so you get a more -- rather than an acute exposure, as it were, you get a longer term.

DR. PORTIER: Dr. Hayton.

DR. HAYTON: Yes, I agree with what Richard Greenwood said. The other thing I think that would happen is that there would be a lower exposure rate because it's coming in the diet continuously. And so to the extent that there are these pre-systemic effects by oral gavage, they could be saturable because you give a fairly large does that gets exposed to that pre-systemic machinery, whereas in the diet it would come

in at a much more -- at a lower level and at a continuous rate, so.

And also the issue that Dr. Greenwood brought up about glutathione pool depletion, that may not happen extensively either. So there's that possibility.

DR. PORTIER: Good question. Any additional comments, questions?

(No response.)

DR. PORTIER: Okay. I don't see any. I think what we're going to do is go ahead and take a 15 minute break before we jump into the big question of 1.9, and that'll avoid anybody falling asleep in the mid-afternoon slump. Right? So we'll be back at 2:45.

(Whereupon, the above-entitled matter went off the record at 2:27 p.m. and resumed at 2:50 p.m.)

DR. PORTIER: Let's get started. We're going to dive into this last question of Section 1, Question 1.9, which we've broken up

into kind of two parts, a risk assessment part and a PK part, and we've got kind of two teams that were assigned to answer those questions.

Just to kind of forewarn the panel, we're not going to move on to Question 2.1 till tomorrow morning, but there are still some clarification questions that the hydrologists and the statisticians have about the hydrology simulations, we we're going to ask EPA and Syngenta to come back up for about a half hour and see if we can kind of clarify especially what those simulations were all about so that we're really certain what they did, and in hopes that that's going to make it easier to us to answer the questions tomorrow morning if we really know what they did.

My goal is to break a little after 4:30, early today. So, you know, in compensation for yesterday. So -- I'm a mean kind of person, but I'm very variable. Right?

So, Dr. Lowit, are you going to read Question 1.9?

DR. LOWIT: Sure. And as you're talking about the next steps and everything else, just a little FYI to the panel, I believe Drs. Cooper, Laws, Stoker and Luebke will be either leaving tonight or first thing in the morning, so you may not see them again, or just for a little bit in the morning -- today at the meeting.

DR. PORTIER: That's a threat. Okay.

DR. LOWIT: Yes. No. Oh, god. They're going home. I'm just going to stop. I'm just going to stop.

So if there are any lingering technical issues that we've -- that are hazard assessment related that we need to rely on their expertise, we're just -- we may want to do that before you cut off today.

With that, I will read Charge Question 1.9. After the April 2010 SAP, the Agency will evaluate the weight of the evidence, what we call WOE, or woe, for

atrazine -- woe is us -- for atrazine by integrating the experimental toxicology data with the epidemiological studies. As part of this work, the Agency will consider the available data on hormonal changes and functional outcomes that may be used as endpoints for deriving PoDs, and that means -- that's what we mean by points of departure that I talked about earlier -- across different durations of exposure and for different populations including potentially sensitive ones.

Important studies evaluating the dose response relationships for hormones involved in the HPA axis are still ongoing. These studies are expected to provide high quality data that can be used to characterize the entire dose response curve. With the caveat that these data are not available now, given the current understanding of atrazine's mode of action, please comment on what would be appropriate endpoints to consider for

deriving points of departure in a possible future risk assessment.

And the second half, given that the duration of the toxicological concern is a key factor in evaluating the frequency of drinking water monitoring, please include in your response a consideration of the magnitude and duration of changes in key events in the toxicity pathway that are sufficient to perturb normal function and comprise human health. In defining the exposure window of interest, please comment on the toxicokinetic and dynamic considerations with respect to atrazine's effects on the HPA/HPG axis.

DR. PORTIER: So you need to roll it back to the first section. And I'll turn it over to Dr. Reed, who has a strategy to answer this question.

DR. REED: Right. When you don't have the answer, ask.

Well, I thought I would give an introduction. As Dr. Portier had said, that

I have a strategy, and he said it was okay. I thought I would give an introduction and maybe, you know, set the stage for input from all areas, that I'm sure you are all storing up this -- you know, something that you couldn't say until now.

As we've been looking upstream, I think, up until this point, upstream to the pathway of toxicity to define the mode of action, or actions, this issue question is focusing us downstream to look at the key events to define endpoints. And so by looking downstream a little bit -- and it doesn't have to be far, far downstream, it's really up to you to define, this essentially connects us back to the whole body physiology and systemic toxicity, or systematic toxicity.

My definition, and you can all, you know, put in your definition, my definition of endpoint is the adverse health effect that can be defined quantitatively with respect to functional manifestation of

toxicological significance, or clinical and disease expression such as age-specific functions that are either compromised, or a functional deficit or over-expression, or perturbation of normal physiological expressions.

So after I invite my fellow discussants to go ahead first, as I said, I would like to hear from every endpoint group about what endpoints within your expertise considerations that you would use, you know, for point of departure for risk assessment. We, early on, talked about this may be the time or the place where the rubber meets the road. If it were so, then I'd like us to all, you know, put our heads together and it would be going forward all-wheel drive, not just front- or back-wheel drive.

The bruntness of the interest that I expect to come out of this issue would really reflect the complexity of addressing the risk of an endocrine destructor with

complex signal pathways, and, you know, their induction, their inhibition, and any other possible mode of action that is non-exclusive, or what was the -- general versus specific to what we're interested in.

And instead of a shopping list, I thought when you give your input, it'll be good to keep in mind the sensitivity issue in that risk assessment. We're looking for the most sensitive endpoint with the assumption that if you have taken care of -- or protected against the most sensitive endpoint, then, you know, the rest of this endpoint will not occur.

And it would also help, now that, you know, this morning you're heard about how these endpoints are going to be used in risk assessment, that if you have some sense of sensitivity in terms of whether humans are far more sensitive than animals pertaining to the endpoint that you're thinking of, then it would be nice to have that information too.

Just as a start off point, you've heard the Agency coming with what they currently have been using, and you have an acute toxicity endpoint, you have the short term, you have the intermediate term, so if you want to give your input in such a -- you know, sort of a duration context, that would be good too. And keep in mind that the pharmacokinetic/pharmacodynamic part will be a separate part that you could put in your input.

So with that, I just want to remind you that -- I can't remember, this morning or yesterday -- I think it's this morning; such a long day -- I mentioned that besides the endpoint male and female reproductive and then we talked about hormone surge, I mentioned this morning that in the older risk assessment we were also looking into some sensitive endpoints as expressed in cardio type of effect: atrial fibrillation, extra-medullary hematopoietic -- you know, in

spleen, that type of thing, and, you know, it might fit in, it might not fit in, but just put a context of that's what you see in the whole animal study in the past where we just don't have the mode of action, only looking at the endpoints. And so maybe that picture will be changed.

So with that, I will pass the --

DR. FENNER-CRISP: I think I've been set up. Long, long ago, far, far away, like over in Crystal City, there once was a time when ATD staff was asked to do a literature search and look for other information in addition to the registration package. They said, what? You want me to do what? And it was really very difficult to have that happen.

Obviously some things have changed for the better, and in this particular case, on an aspect of one chemical there are a lot of additional data that have been assembled and reviewed. I think yesterday or the day

before, and a couple of times today, though, panel members have expressed some concern about perhaps premature dismissal of some of that information. There was some of that conversation when talking about the neurotoxicity, there was some of that conversation yesterday when talking about some of the cancer genotox data that were part of this review. So I think one needs to be mindful of that.

And in a comment today about -- we're really starting from scratch in the reassessment. I interpret that to mean that we're going to take another look at everything we have, not simply all the stuff we've concentrated on for the last few days, which is really just one category, or all related to kind of one category of information. There's a whole lot -- if somebody were to bring in the registrant's submissions and place them in the middle of this four-sided table, the pile of paper would represent a lot of trees and it

would probably exceed the height of the table. So you have to be mindful of that.

Okay. So one of my comments would be, remember that if one is actually doing a reassessment from scratch, that one needs to at least take a cursory view of all the toxicity data from all the studies that are part of the registration record and all of the supplementary stuff that has been acquired since, and revisit those and reconsider them in the light of any new data that may be available in these other domains, because it might well be that something we didn't know about, endpoints, particular endpoints may have come to light through other work, not necessarily atrazine specific. So that's my first point.

In this particular case, we have a number of new data sets and new parameters with the further exploration of the mode of action related to reproductive effects, which also require a revisit, and we've talked about

that, of the prior assessment and make decisions on whether or not to use what was previously used in the risk assessment, or whether or not some part of the new information will become the focus of the risk assessment for characterization of point of departure, et cetera.

I would offer my opinion that the appropriate endpoints for consideration for deriving PoDs going forward would be the data sets at each key event now characterized in the mode of action. When we once had a set of six or seven for the mammary tumor, and some of those were overlapping with what was thought about with respect to reproductive and developmental toxicity, now there's another whole set of data with -- related to HPA axis.

So given the definition of key event, presumably there are data that are amenable to evaluation. They referred early on that this is being done in the context of the IPCS mode of action, human relevance

framework, and the Agency's guidelines, which describe a key event as an empirically observable precursor step that is itself a necessary element of the mode of action or biologically-based marker for such an element. Meaning you have empirically generated data that can be modeled or evaluated in some way.

So I would submit in this particular, given the apparent importance of this chemical and all of the effort that's been put into its risk assessment, that it's time to do a benchmark dose analysis for everything on this particular chemical, and you're beyond the point where you can just pick NOELs and do things.

So I would submit that at this point in time one should be doing BMD analysis on every key event, both sides of this new picture, older picture and newer picture, HPG and HPA. Obviously modeling it to the same value, what do you use BMDL, BMD10, whatever it is, and then compare as you do in the

cumulative risk assessment. And from that, that will inform you which one among those data sets, if any, should be the appropriate ones from which to define points of departure and proceed with the risk assessment.

One point that's probably more related to our PK folks' comments is that it's been pointed out several times, and I'm looking particularly at the NHEERL data with the parent and the metabolites, that not all four of those substances necessarily do the same thing when tested in the same system. And, in fact, there are some examples where the parent in one or two metabolites may exert a particular effect and the other one doesn't at all. There are a couple of studies that have been pointed out. So how does one account for that when trying to do the risk assessment. I think one needs to be mindful of that on the plus/minus examples.

So I'll leave it there and delegate to somebody else to do be the sticky

for the moment.

DR. PORTIER: Well, before you run, because as I was making notes, you made a lot of references to key events, and I wonder, in your mind, what are the key elements, I guess is the word.

DR. FENNER-CRISP: The key -- no, key events.

DR. PORTIER: Key events. What are the key events in your mind that they should be looking at, or what are the ones that are going through your mind?

DR. FENNER-CRISP: Well, I'm not disagreeing with the characterization for the key events that previously were identified in the early stages of the -- well, the mammary tumor mode of action in the beginning stages of looking at the repro and developmentally effects.

So we've got -- I think there are eight in there, but the last one referred to the genotox thing, so it's essentially seven.

It's the precursor events plus the endpoint of concern. If you don't have sufficient data to do a dose response analysis for any one of those, you know, it's got -- it's less convincing than if you do.

And now and looking on the other side of the -- the other axis, there are four key events. So the question is, are there empirically observable, quantitative measures of those key events that could be looked at as potential data sets for PoD identification.

DR. PORTIER: Okay. Dr. Reed.

DR. REED: Could I get a clarification out of -- when you mention doing benchmark dose analysis on every key event, and -- were you thinking of treating each benchmark response -- or benchmark dose with the same benchmark response like whatever percent of response, or would you be open to a different interpretation in terms of the significance of that response?

DR. FENNER-CRISP: Well, the most

useful thing would be to be able to model to the same point so that you can make some comparisons. But sometimes, depending upon the nature of the data, one can't.

DR. REED: Okay.

DR. LOWIT: If I could just jump in for a second there. I think in this context there are two different but important uses of the benchmark dose techniques. There's -- I think the way that Penny's thinking, in a more -- you do a mode of action framework in a temporal concordance dose response concordance analysis in the same way that we've done for a couple of others, like cadodylic acid I think is a good example, where you take the data from the key events and you select a common response level and you evaluate all of the events at the same response level to look at the pattern of the sensitivity of those endpoints at one common response.

But that's a different question of

using those data as a regulatory endpoint because then I think you have to bring out a more robust thinking of changes from background or historical changes and the relationship of the biological significance of one key event to the other. So I think you could use the benchmark dose differently in that way.

DR. FENNER-CRISP: The reason I offered that suggestion is that it is quite possible and quite likely that the shape of the does response for key event A may not be identical to the dose response curve for B, and looking at NOELs you may think they're in this order, but modeling them they may shift the order, and one has to take that into consideration when moving to the risk assessment.

DR. PORTIER: I guess the other presenter is Dr. Krishnan.

DR. KRISHNAN: I'm going to be pretty much repeating what I've said, but

let's see how much of it is the same and how much is different.

Well, with respect to what endpoints to base it on, we've heard a lot of good discussion about the relevant endpoints to be considered in the context of hazard identification pretty much this morning. And then, you know, based on considerations of relevance and mode of action, you'll be looking forward to as to which one of those will go down the path of further analysis.

I see kind of two scenarios. I think we already heard some of that. First would be the PoD being derived on the basis of key events, particularly as related to the reproductive function and depicted by picture 3, as you alluded to. That's good as long as it's the one MOA, or that set of MOA that determines the critical outcome. And one MOA may not fit all the critical out of a lot of several endpoints we talked about.

So for some of the other

endpoints, I guess the alternative scenario would be deriving PoDs based on the outcome data itself and not necessarily based on the key events being a part of the mode of action framework that remains to be seen.

Technically I don't see us being able to say which endpoints will have a lower PoD, and that's unless these analyses are actually done, because in part of kind of dose measure that's used on the X-axis will also determine -- and might change what one might see as being sensitive or less sensitive based on external dose only. There are examples to that, in fact.

The development of PoDs based on biomarker levels or perturbation levels would be relevant as long as the relationship to the functional outcome is established quantitatively. You already heard some response to that effect. So the PoDs are based on, for example, hormone levels, so the consideration of the homeostatic and

physiological considerations as a basis of benchmark dose analysis would become important rather than using the traditional 5 percent or 10 percent change, which was also kind of unpolished, to induct in the comments before me.

In this regard, one thing that I thought would be nice to see varying thicknesses of the arrows, you know, depicting the proposed mode of action in Figure 3, or as you go, as you move forward. In certain cases the thicker arrow, that gives, you know, a stronger weight of evidence whereas the other ones could be weaker, it could be a dotted line thing or it could be a solid, things like that.

Or, in other words, for specific toxicity perturbation based, you know, analysis, it would be relevant to identify the availability and competence of the data for each of those critical events, for example, from which the analysis could be done. So the

ideal -- the desired data would be one, but what's available might be different. So honoring them in terms of, you know, on the basis of consideration of data availability and their competence, the strength of the relationship to the functional effect for any of the key events that you would get in such an analysis, and also the strength of the model fit, which will be done at the end of the BMD analysis, rather than just basing only on the response modeling.

And in considering the PoD derivations, I also think that it's important to give due consideration to the internal dose as the dose measure to replace the administered dose given the fact that there's some possibility of situations where you may have some glutathione depletion or first pass affects influencing the nature of the dose response.

And in this regard, without getting too much into the next question, I

think the measures of dose in deriving the PoD will have to be somewhat related to the total chlorinated forms, it appears, even though we heard more about the two -- the metabolites, the dealkylated ones that do not affect the hormone levels, whereas some of the other studies from Cooper, et al., that identified the DACT, you know, in some evaluations of both male reproductive functions. So I didn't see the connection between the lack of effect of DACT on the hormone levels, but effect on the whole animal levels. So maybe we ought to look at clarification on that aspect.

I just have a couple of sentences to go through. Because in those studies there were -- some atrazine-equal end doses were provided for DACT, as for the other metabolites, which then kind of, you know, leads -- possibly leads us down the path of atrazine-equivalent doses at dose measure, you know, internally, which doesn't just represent the atrazine but as well as the other forms,

and there's an equivalence that's derived. I'm not saying that's appropriate, but it does make sense to me when I went through those.

In that regard, PBPK models, might be able to provide -- depending on the level of the maturity, they could provide that kind of an appropriate dose measure, because one of the papers did present some of the total chloral forms of the atrazine.

So the animal model in itself can provide an improvement of the X-axis in deriving, or getting a better handle on the PoDs. And if there's a human model, that means subsequently, you could, you know, even think of replacing the interspecies factors.

So I'm not getting into the risk assessment at all. I'll just stay -- pull back. I'll erase the last word that said -- answering de facto. I'll leave it at that, and if Cooper, et al., can clarify the determination, the equivalences for DACT, I would appreciate it, and whether they see a

connection or no between the hormone levels and the hormonal effects.

DR. PORTIER: Dr. Cooper.

DR. COOPER: That's an interesting question because -- I want to restate it just to make sure I address it properly. If you look at all the data with DACT versus the other metabolites, and you look at the reproductive axis, you look at the effects on LH, we've done a pretty complete assessment in the female on the LH surge with those four different molecules.

And what we see, or what we have seen over the years when we were naively dosing equal amount of doses, you know, we thought we were delivering the same number of molecules or whatever, but when we eventually went to look at the internal dose, and we were fortunate enough in some of the more recent studies to have assistance with looking at different -- the four -- the three metabolites plus atrazine in the blood, what found was

even though we were naively gavaging with equimolar doses, we were getting considerably different concentrations of the different metabolites in the blood when we dosed.

And we've already addressed that, very little atrazine, per se, at least in the time points we looked at. We haven't done, obviously, a complete series -- time series of measurements.

But DACT was very high. That's number one. So the internal dose of DACT is very high whether we dose once or four times, and given the DACT was really active, we were sort of surprised that such high concentrations were present. But secondly, the amount of DACT necessary to inhibit the LH surge was higher even yet. So now when we do a dose response, and we don't have this complete, but we have it piecemeal in different studies, that we have an established NOEL separately for DACT, we have some preliminary data it might be around 100, which

is surprising or interesting because it's -- in itself, if you dose by itself, it still has on the surge the same type of effect that you see with atrazine, per se, or the other two intermediate metabolites.

So my feeling is that that particular metabolite, on the LH itself, is a lot lower. And then if you go to -- but it's still effective and I think that's the point, the sticking point here. You know, in a perfect world, you know, DACT, if you were hypothesizing an adrenal involvement, it shouldn't be active at all, and so on. So that higher dose we see effects.

And then of course when you look either in vitro, and this is the thing, it's very interesting about the comparisons is that when you look in vitro and the effects on adrenal, then DACT seems to have very -- even more minimal effects, if you will. So that's the situation.

I think what we're dealing with

here of course is more than one -- like my mother used to say, more than one way to skin a cat, as that you can effect that surge either directly using DACT, or with some of the other compounds there may be a much broader series of events that take place. So even though it doesn't line itself up nice and neatly, I think we're still dealing with compounds that all eventually will knock down the amount of luteinizing hormone secreted.

And did that get at what you're after? In other words, I agree. Additionally though, what I was more interested in was the study of Laws 2003, which was on page 31 and 32 of the document.

DR. COOPER: Of the female?

DR. KRISHNAN: Yes. And for male it was the Stoker 2005 --

DR. COOPER: Right. They both were --

DR. KRISHNAN: -- on page 34.

DR. COOPER: -- in Stoker paper

she dosed again with 21 days in the male which -- 31, I'm sorry, the female gets -- 31 days, that's a month of dosing, daily dosing, so again, even though in that one we had a LOEL th was down in the range of the other metabolites. I think 30 days of dosing is different than the comparisons that I was making with the shorter term.

And, Susan, the female, the NOEL for DACT, did you get a NOEL in the female for DACT in the pubertal? I know you compared propazine and one of the other intermediate ones, but not -- she's frantically -- I can tell frantically searching through her document and saying that DACT was also examined and she found that DACT --

DR. PORTIER: Let her speak for herself.

DR. COOPER: -- was delayed vaginal opening in these animals at a dose of --

DR. LAWS: I've got --

DR. PORTIER: Identify yourself.

DR. LAWS: Susan Laws, EPA, and the no effect level for DACT was 16.7 milligrams, which was equivalent -- equimolar at the time to atrazine 25. That was the no effect. So probably the LOEL was 50 for DACT.

But one for the things that I wanted to mention, in the 2009 papers, although in Laws, et al., it's one dose, but -- and that, in Tables 4 and 5, we had the plasma levels of the parent compound and the metabolites on their dose of atrazine. And that's what Ralph was mentioning early -- and we also have -- when we treated the animals with DIA or DACT, and we see that in the first we have a dose -- I mean a time course from five minutes to three hours, and you can see the progression of atrazine is essentially the same in vivo, and then the progression to the increased amount of DACT.

And in Fraites, et al., she doses four days and she saw that DACT was --

continued to accumulate. So it may be in the pubertals that DACT does continue to accumulate some in the plasma, which may actually get to a point where it's causing these effects, because the pubertals are a 21- or a 30-day exposure period versus the acute things.

DR. PORTIER: So I think what -- Dr. Reed wants to open it up now for anyone else to discuss endpoint. Anyone else? Yes, Dr. Greenwood.

DR. GREENWOOD: I just wondered if I could ask a point of clarification. When you looked at the accumulation of diamino chlorotriazine in the plasma, was it free or bound, because if it's bound to the serum albumin covalently, it's not going to be doing a lot metabolically.

DR. LAWS: I'm sorry. This was in the plasma, and it's total -- as far as I know, it's in plasma, not serum.

DR. PORTIER: Dr. Horton.

DR. HORTON: I have a question about the definition of a key event, or maybe I just misheard something between what Dr. Fenner-Crisp and what Dr. Krishnan were saying, because what I understood from what Dr. Fenner-Crisp was saying was that a key event has to be part of the MOA, but what I thought I heard Dr. Krishnan saying was that in looking for points of departures, that some of the key events may not belong to the current mode of action.

Were you saying that, while we may see some key events that, you know, aren't necessarily part of a mode of action, or were you simply referring to the breast cancer, or the mammary tumor mode of action, but they may be part of the new mode of action that's the model on page 3? Or what did you actually mean?

DR. KRISHNAN: Okay. In terms of the mode of action framework, the picture 3, what I indicated was maybe there are some

connections and lines that are -- that could be strengthened and some that can be weakened rather than considering all of them as being equally, you know, supported by the current data, that something they thought could be then.

On the other hand, for specific endpoints that are used in assessments, a single mode is not going to -- a single mode of action framework might not end up explaining all of the endpoints and outcomes for which, you know, you're going to base the assessment on. So, you know, it's not like, you know, going through the same mode, the house, and then a lot of the outcomes come through, that probably is not a realistic -- and so in that case --

DR. HORTON: Okay.

DR. KRISHNAN: -- they might do some analysis even without going through the detailed mode of action if they don't have --

DR. HORTON: Okay. Dr. Horton

again. So in this case now the house has some out buildings, we have a barn and -- okay.

DR. PORTIER: And I was -- I've been following that, I'm wondering what you think some of those out buildings might look like, because we've been talking about kind of non-hormonal process, repo/developmental events that are neuro or whatever.

DR. HORTON: Okay. In that context, the neuro effects that we were talking about are just in the attic because they're on top of the regulatory system that's part of the HPG/HPA system. And so they are built on top of the regulation of the reproductive and the adrenal system because they are part of the system that regulates that, in the hypothalamus and limbic system.

DR. PORTIER: One question. Is it possible to draw a picture of that? I mean or add to the -- add to Figure 2 to really explain what you're trying to get at.

DR. HORTON: I can do that.

DR. PORTIER: I just -- I'm a visual guy.

Yes, Dr. Fenner-Crisp.

DR. FENNER-CRISP: Maybe another thing that would clarify what Dr. Krishnan said was, not only might you select from the key events related to the mode or modes of action that have been postulated for reproductive and developmental effects, you may also, by virtue of time and exposure duration choose to select a totally different outcome for which no mode of action analysis has been developed to this point in time.

And if you look at the body of data related to this chemical or any other pesticide, there are whole lot of other things that get measured in apical studies for which mode of action analysis may never get developed, like body weight changes in a chronic study, or things like that.

So it may be appropriate to go to some of these other data sets by virtue of

subpopulation, duration of exposure, et cetera, et cetera, and use that data set as the basis for your point of departure. And you can use any of the stuff we've been mulling over for the last two days.

DR. PORTIER: This is Ken Portier. But my understanding is that probably was looked at in the 2003 risk assessment, and ruled out in favor of the LH surge --

DR. FENNER-CRISP: Except that I offer the admonish that there may be things known about some of these other endpoints now that weren't known back then that you may want to take a second look at.

DR. PORTIER: Dr. Lowit.

DR. LOWIT: With the caveat of what Penny just slapped us on the wrist about, I think at this point we have a little bit of confidence -- I don't want to go too far -- that the precursor events around the hormonal changes are more sensitive than the functional outcomes. I'm not aware of any of the

functional outcomes that are more sensitive than are the hormonal changes. And someone kick me if I'm wrong. There are a lot of people behind me that probably know the data.

Ralph's arguing yes, so may I -- that's part of what we'll ask ourselves, but I think our driving theory right now, and there's -- we haven't seen anything to get off that train, but if we see something, we will, is that the hormone changes, or the precursor events will be -- we expect them to be more sensitive than the outcome data.

DR. PORTIER: Dr. Akana I think was next. And then Dr. Hayton.

DR. AKANA: Okay. I'm not positive I've got a grip on the idea, but I'm going to throw out an endpoint, and it's just a theoretical possibility. And that is actually continuous recording of body core temperature. Now normally you think, okay, core temperature, you know, it's core, it doesn't move that much, but actually with the

nice little implantable transponders, you can demonstrate beautiful basal circadian rhythms in temperature, which are reproducible.

The stats people will love it. It generates hundreds and thousands of points for you. It's got maxima and minima, it can be modeled, it's sensitive to glucocorticoids, it's sensitive to food intake. I don't know the data in females, but I'm hoping it shows beautiful cycles you can follow through. One of the beauties is that you can give -- you know, once the animal recovers, you can give your acute dose and keep continuing, and you're collecting online data.

So it's not so great for the immune measures that mostly would catch accrued temperature, but I'm wondering how many people could be happy with that kind of endpoint. Oh, and then it covers catechols.

DR. HAYTON: Yes, Bill Hayton.

DR. LOWIT: One more quick -- with quite a number of these you'd catch locomotor

activity which you can, from the same transmitters, so that can be -- usually home cage is really valuable, or depending on your model it could be other locomotor measures, which also have -- can be verified with photo detectors.

DR. COOPER: So let me ask you the question then, if you did this and we have the radiotelemetry and it's accessible to us, how readily accessible, I'm not sure of, and there's some of my colleagues who are looking into some of these approaches, but we have -- I could envision three outcomes, and I'll run them by you and you'll see the dilemma that you're suggesting.

The first outcome would be we see a difference, and we see at just about the same dose response that we see -- I'll pick another endpoint, the surge, okay. So we've talked about that. How do I establish a functional link on that one?

I mean this is really what we're

getting at a little bit. You've got another endpoint that changed. Okay. Granted. And it's a measure one, a repeated one. But we have to establish a functional link. And there's the literature there, I'm sure, there's a literature on everything.

But the other one is it comes in at a lower dose. How confident can we be that that's indicative of an adverse outcome functionally linked to delayed vaginal opening or mammary gland tumor development and so on? And then the third one is no difference, I guess, or high dose difference because I doubt that you'll see that if we get involved with any of these measures you can be pretty sure you'll see effects.

So I mean the question that we really have to get at is, with what we have now, how can we proceed in terms of taking the best estimate of what the real predictors of adverse outcomes are in the data sets we have? In the previous SAP they agreed that the surge

itself was a good key event that could be used at this. We were concerned that the adrenals may offer additional lower doses or something like that.

But I would doubt -- if we took Penny's approach, I don't think we'd see a difference in the benchmark because I think they'd probably be pretty -- between the, say, the surge and the ACTH or cort or whatever -- however you want to evaluate that.

So, again, I think we're really interested in your comments on which is the better of what we've got right now knowing that we don't have the Cadillac we'd love to have.

DR. PORTIER: Dr. Hayton.

DR. HAYTON: Thank you. I just -- I'm still puzzled. The question, 1.9, what would be appropriate endpoints to consider. I've been trying to write something down here, and I don't think I've quite heard it yet, but it -- we're talking about HPA/HPG signals.

Right? Okay. And then downstream sequelae to those particular signals. Okay. Thank you.

DR. PORTIER: So I think the LH surge is still in there. Right? And there's some others on the HPA axis that are cong to come up that we're looking at. And the question is, is there anything beyond that that's in that part of the box. Right? And, Dr. Horton.

DR. HORTON: Okay. Two things. One is I think we need to redraw the figure to be able to figure out what some of those key events may be to ask what the endpoints are, because I think that's what you want to know, is what are those endpoints, and those endpoints have to be defined by looking at that box.

And with respect to the functional issue of the rhythms data, how you interpret that functional outcome will depend on specifically what characteristics of the rhythms are disrupted and how they changed,

because if it's increased activity, you've got one functional outcome; if it's decreased, it's a different issue; if it's a delay in the rhythm, as I've heard some suggestion that there's a delay in the onset of the rise, that means a different thing. So there is actually a lot in terms of the functionality that can be interpreted from looking at changes in circadian rhythms. And those things do have a great deal of impact in terms of looking at metabolism and health outcomes.

DR. PORTIER: But what I keep hearing is that the early endocrine things, like LH surge, are going to be precursors to any of those functional -- if you look at Figure 3, the functional -- or further down the line, and I think the argument is that there's going to be less sensitivity to that and to these things that are in the pituitary box --

DR. HORTON: Well, it's --

DR. PORTIER: -- or in the CNS

box.

DR. HORTON: Yes, except that you can pick up -- no, you can pick up circadian changes in males but you can't -- you're not going to get an LH surge in females, and so therefore if you're concerned about reducing animal care costs and use of animals, you can look in both males and females. The advantage of looking in males is you don't have to worry about a four-day cycle, so you've reduced by a factor of four the number of animals that you're working with.

So -- although you do have to deal with some of these issues that seem to be sexually differentiated. And also you can look at different ages. And so you do have some different considerations to take in mind whether you're looking at a circadian marker rather than an endocrine marker.

DR. LOWIT: I think there's just one point that I'd like to make before we sort of keep moving, is that what's unique about

Question 1.9, that's different from 1.2 up to 1.8 is that if you go back from a few days ago, the rubber meets the road analogy, this is where the rubber meets the road, and the Agency has to start making some choices soon. Soon, you know.

And so to the extent that we can -- you can, in your mind, as hard as it may be, separate yourself, put yourself in our shoes for a minute, that the Agency has to make some choices very, very soon, before September, in fact. So between -- in the next four months.

So the things that we have in front of us, that you have, and the seven to ten things that Liz listed up on her slides a few days ago, are all there is to make those choices from. Okay. With respect to these non-cancer endpoints. And so this is a science question because it's getting at the things to think about as we make those choices, but this is not a research open-ended

kind of question.

DR. PORTIER: Dr. Fenner-Crisp, and then Dr. --

DR. FENNER-CRISP: Yes, Dr. Krishnan referred to it. One thing that currently doesn't exist, that I'm certain will before you make your decisions, that would have been -- that would be very helpful to have, is the framework analysis prepared in parallel to that which was done for the mammary tumor mode of action. And then one could -- it would speak to all the things that were talked about: about how thick the arrows ought to be from where and where, what's the credibility of the data set, you know, and all of those kinds of things, the temporal relationships, the dose -- you know, all that stuff.

Were that to exist, it would be much easier for this panel to answer your question. And I know you will be preparing it.

(Laughter.)

DR. LOWIT: Thank you. Yes. Absolutely. Probably starting next week actually.

DR. FENNER-CRISP: Yes.

DR. LOWIT: Our assay is halfway done. All right. But some of the studies that Cooper and company and Syngenta are doing are important in that because they provide some of the causal links, and some of the Handa data from the other day really establishes some causality, and there's a number of dose response studies in there that are driving the doses five and below, which gets out some of the comments.

So it was really almost impossible to do a few months ago, but three months from how we'll actually be a lot --

DR. FENNER-CRISP: Well, I appreciate that, and I wouldn't wish to suggest I had an expectation it would exist yet. But the point is that eventually, before

you make a final decision, you have to have created that document in order to best present the argument for selecting the things that you select. That's all I'm saying.

DR. LOWIT: Yes. And Dr. Portier, I think, speaking for the team, at least in this first part, I think we've heard what we've sort of expected or thought, and I don't want to beat it to death. I think the really interesting part is the second part.

DR. PORTIER: I was going to acknowledge Dr. Reed, and then ask her to summarize so we can move on to the second part.

DR. REED: That was pretty much why I'd raised my hand. I think we heard enough to move forward. There's certain things that we wouldn't know until, you know, you get into the analysis, and so that's my --

DR. PORTIER: So let's pop up the second part of the question, and I'll turn to Dr. Krishnan to talk about the issue of

duration. Right? And some of the PK issues.

DR. KRISHNAN: I'm not going to make it interesting, if that's what you're looking for.

(Laughter.)

DR. KRISHNAN: But I have some able colleagues by my side who might try that. I'll put in some of my thinking, and then I'll leave it up to the colleagues, and then I'll come back with additional comments.

The question essentially relates the duration of toxicological concern and the frequency of drinking water monitoring that we've been talking about on and off, you know, at least yesterday. And in defining the exposure window of interest requested, that's the comment on the pharmacokinetic dynamic considerations with respect to the atrazine effects on the HPA screening axis.

I think initially as we think about the problem in terms of acute versus chronic, or repeated exposures, you can

always, you know, somewhere draw the line of intermediate versus chronic, because often the continued exposures get us to what is called as a steady state. So knowing this characteristic of this chemical, I don't expect to -- at least kinetically I don't expect to see differences between intermediate versus chronic duration.

So if we consider acute versus chronic scenarios, normally when we protect against chronic effects, protection against acute effects is automatic, and the vice versa is not true. So then we also saw some of the box and whisker plots that showed increasing space as you went from chronic to acute.

I'm not going into any specifics of the effects, critical effects between acute and chronic, but in general terms for acute effects you tend to think of maximal concentration as the driver, maximal concentration internally, not in the plasma or the critical tissue. So it's a question of

whether we exceed a threshold maximal concentration, for a C-max, and for how long. That's the question with respect to acute scenarios.

Then for repeated exposures of chronic, it's often the inner greater internal exposure, or the average daily exposure. That's usually a useful measure. Those are some for the generalities, even though you can always come up with exceptions.

Now, when we think about the frequency for drinking water monitoring and the plots of the water concentrations that we saw, you know, there's peak who appeared for a day or a shorter duration and there was some trials and so on mixed with it.

But relating that, or viewing that in the context of toxicological concern requires that we think of the continuum of events, meaning that from the water concentration that we see was on the pictures, the next one that I would think of is the

exposure pattern, what is the rate and route of contact. Now it might take just a half a cup of water versus a bottle of water. That's a factor of 10, and then you're talking about the peak and then another -- or differences there.

So the exposure -- the rate of uptake and then the route of exposure, whether it's only oral, or dermal and other routes, whether they're relevant or not. In most drinking water scenarios, those are considered. I'm not -- it seems to me that the dermal absorption is much less, but I'm unable to give an quantitative estimate based on permeability coefficient. I don't know if I missed the permeability coefficient. I haven't seen it, but those can be estimated. EPA has some methodologies available.

So from there, the exposure pattern, or the uptake pattern, and then we to consider the pharmacokinetics which is the ADME, the initial step of rate of absorption

would appear to be the limiting factor, while it's relatively a lower rate of absorption as it seems from some of the papers we have seen.

So depending on the quantity and the frequency we take, there's the rate of absorption that takes into -- that gets into the play. That's one of the reasons why I feel that an integrated measure of exposure would be more useful rather than concentrating on any of the individual peaks and trials. And also when you, you know, integrate and use an AUC for -- in this context, either internally or externally, the area under the curve of concentration versus time, that also deletes out any -- possibly deletes out any impacts or concerns about specific peaks, or very sharp duration.

Also, we heard some -- in terms of the pharmacodynamics aspects, I have done the environmental concentration, the uptake, pharmacokinetics, even though I haven't gotten into all of the details, which my colleagues

will, and then in terms of the pharmacodynamics as well. And Dr. O'Byrne talked about it this morning. I thought you emphasized the importance of the sustained chronic alteration of the precursor levels, especially when you talked about the cortical levels this morning. That's what really determines the impact on the LH surge, if I didn't actually put it.

So in total, considering the continuum of those events, I'm coming back to the question of duration of the tox concern versus the frequency of monitoring. My initial take -- even though I can learn from people and change my mind later on, my take is that, you know, monitoring the way we have seen, like a weekly monitoring interval over a three-month period, or a certain period, I think will serve the purpose.

Since we haven't seen, or at least I haven't seen a study where it really strikes by saying that while the critical window of

exposure is one day, or between day 3 and day 4 of -- during a gestation period or something, then it really puts you under the pressure to have to essentially monitor within that kind of a time frame all the time, because you don't know who will be in that time frame and when.

Since that doesn't seem to be the case, it appears to me that the area under the curve kind of approach would be sufficient at these intervals, and the Agency's may not be as sensitive to very short term fluctuations. And at some point, some kind of sensitivity analysis might be of use. Now once we do determine a PoD, we already have the dose measure, you can always relate that to essentially water concentrations to these temps.

And I've tried to ask the question of what is the most sensitive parameter that really drives my, you know, dose measure that I'm using in a risk assessment, or in a dose

response curve for humans. Is it the uptake, my peak, and so forth? So that would also give some -- well, I think will give some added confidence in terms of use of, you know, the area under the curve kind for a measure, even for the environmental concentrations.

I think with that I'm going to yield the rest of my time to Dr. Greenwood from the County of Portsmouth in the UK.

DR. GREENWOOD: Okay. I just want to address, because I'm sure everybody's read what's in the white paper, but I just want to address what I think are one or two important points that hit me between the eyes when I was reading some of these papers.

One of the things that came up was that in rats we get this binding to hemoglobin and to plasma proteins. So if you're looking at -- want to look at the level of free diamino chlorotriazine, because this is the stuff that binds rather than the other metabolites, or atrazine to any great extent,

then it's certainly going to have an influence, or should have an influence on the interpretation of the levels that you're measuring in blood.

And some of the other studies sort of acknowledge that this happens, and then forgotten about it when they come to interpret the profiles, particularly if you're trying to get a handle on what is a critical exposure of site of action to a particular toxicant, whether it be atrazine or one of the metabolites. And it just seemed that when you look at some of these profiles, that in some of these studies, because they're high dose maybe, I don't know, but because they're high dose we're getting really not very much relatively of the glutathione derivatives which are relatively -- seem to pharmacologically inactive, as you might expect, were compared with the oxidative metabolites of atrazine and atrazine itself.

And certainly the diamino

chlorotriazine seems to be present in some of these studies at about a factor of 100, more than any of the other metabolites. It really is, after something like 12 hours, is the predominant metabolite. Atrazine seems to be peaking after a very few hours. So this just seemed to be the major metabolite.

And what's more, when people started to dose this on its own rather than atrazine just to follow the kinetics of it, it's got a very rapid absorption, and a lower level of elimination. It's got a text book sort of profile for that sort of compound. And it does seem to bind -- because it binds to cysteine, it does seem to expose cysteines. It binds to a wide range of proteins.

Now, obviously there's a large volume of albumin in the bolus, predominant -- in the plasma it's the predominant protein and it does bind to one particular cysteine in there. But it also seems to bind, when we flip to the later studies, to a lot of

different proteins in, for instance, the pituitary. But, again, there's absolutely -- I have no idea what the toxicological significance of that might be, and how much of the -- like how much of that protein is removed, how quickly it's replaced, if it's just -- is it detected as being non-functional and does it replace. I don't know.

So it could be an important factor, not just for the pharmacokinetics, but maybe for -- it appears that this stuff is inside certain tissues, but if it's found in there, then it's not available. But is the binding itself toxicologically significant. And I don't think at the moment anybody has any idea.

So I think this diamino chlorotriazine is really something that can't be discounted, mainly because it's in there in such large quantities and because it does bind covalently to any cysteine that's in the right sort of conformation.

I think the other things that came across to me, there's a study by Carroll, et al., where they've compared the human and rat, gut and uptake of it, and it did show that the -- I think I said earlier that the absorption is very similar in the two. There's quite a high correlation coefficient, in fact, a coefficient determination of .8. But between the bioavailabilities because of this difference in metabolism in the gut, they R squared value goes down to about .3.

So it's -- it think there are species differences, and although you might predict in terms of absorption what goes on, and you'd probably be fairly reasonably accurate going from one species to another, in terms of bioavailability you may be a long way out, and it's the bioavailability of course that's the most important thing that we need to look at. It's no use something being absorbed if it then gets wiped out in the first pass of metabolism.

So I think, again, looking at some of the studies, some of the models were dismissed because there weren't data to test them and so on, but I think that the model fitting process gives us some take home lessons, I think, about what are the more sensitive parameters in the model and gives us some idea of what would make a big difference. And in most cases, what seems to make a big difference to the exposure is this metabolism, either in the -- by entry sites or by the liver in the first pass of metabolism.

And that's why where we -- when I was asked earlier about implants where you avoid some of that metabolism, I mean eventually it's going to meet those same things because the gut gets a blood supply, it doesn't just have a blood supply taken away to the hepatic portal vein, it also needs an arterial blood supply. All of the tissues come in contact -- once it's in the blood stream, every tissue in the body comes in

contact with it, target tissues and non-target tissues.

So, again, I think some of these issues need looking at because I think what's important is, when you're looking at trying to get a dose response code, is what is available, what is available for interaction with the site of action, because if it's bound up somewhere, it's not available, it's covalently bound to an albumin.

I think I'll leave that there. I think those were the main points that, when people are looking for dose response relationships, they may not always see one, and it could be because you get differential metabolism and you get different pharmacokinetics at high doses and at low doses. And I think one of the other panel members may have something to add to that, so I'll leave it there.

DR. HAYTON: Bill Hayton. Just a few comments to summarize my report I'm going

to turn in. The first thing is that after oral gavage, that the -- in terms of kinetics, the hormonal perturbation occurs very quickly after atrazine administration. I think it's the Fraites papers shows within in 15 minutes. Even though it's a fairly large dose of atrazine, we know from McMullin's study of kinetics, that probably most of the dose is still in the GI tract, so it seems like it doesn't take all that much atrazine hitting the pre-systemic metabolism machinery and then whatever goes on into the systemic circulation. Right away it triggers hormonal perturbations in the HPA axis.

I think another thing that's important about the kinetics is that atrazine and its metabolites are not persistent. They seem to be eliminated fairly quickly. There aren't really good estimates in the rat of half-life of the individuals species, I didn't think, reading the PK papers. But just looking at the overall half-life of

radioactivity, something on the order of about 10 hours in the rat, if you scale that to a human maybe 24 hours, it would be three- to four-fold longer typically.

Anyway, what this means is if you stop -- if a person is exposed to atrazine in drinking water, you get rapid onset of effect on the HPA axis. If you stop administration the effects -- it seems like the direct effects have to go away fairly quickly because of the relatively short half-life.

So those are some of the things that I think that we know. If we ask the question what's the lower limit of magnitude and duration of perturbation of that hormone signal that produces functional outcome disturbance, in other words if you say that the signal is just a precursor to a functional outcome, adverse event, I don't think that's been characterized. I don't think we know, you know, how much perturbation of this signal and/or for how long.

And this was a question I asked Dr. O'Byrne this morning about, you know, the acute versus the chronic and I think the answer was it's the more chronic exposure is probably the more relevant exposure. But I think that's something we don't know.

And then finally, I think the exposure window of interest is not readily definable with the data we have now. Acute perturbation seem to be less relevant than chronic perturbation, and I think without the signal functional outcome relationship being well defined, it seems prudent just to use the lowest observed adverse effect level that perturbs biosignal as the point of departure, or as the endpoint to define that.

DR. PORTIER: Dr. Greenwood.

DR. GREENWOOD: I'll just pick up on one point. And it's difficult to get a feel from these studies which are using oral gavage because of what Dr. Hayton just said, that a lot of the dose is still actually tied

up with the bolus and it comes out slowly, probably in the intestine, over a period of time. I think the whole dose eventually will be absorbed, but -- or most of it, but it's very difficult to try and get a feel for what would be the pharmacokinetics if it was in the drinking water.

And the problem is that, you know, if you start thinking about taking in lower levels in the drinking water, then you've got to detect it, and if you dilute that by all the tissues in the body, then you've problems in the detection. And that's a real problem. So it's very difficult to see how you get a more realistic model of drinking water intake because of these problems of level of detection.

But at some time -- conceptually I find it difficult to go from what's going on with oral gavage and what would be going on with intake throughout the day at lower levels in drinking water. And I think, you know,

you'd probably get a different picture in terms of metabolites and everything else.

DR. PORTIER: Dr. Schlenk.

DR. SCHLENK: Yes, this is very interesting reading. In terms of the kinetics, I think, particularly since most of the doses are all fairly high dose experiments that are primarily oral, and I think it really lends itself to a PK -- PBPK sort of analysis, I think it's perfect because I think, particularly from the metabolism side, you could argue that there's tremendous differences between high dose oral exposures and low level chronic exposures, just from a KM perspective in terms of the P450s that are involved in metabolism.

One thing I would note before I forget, actually I wrote it down, if for the non-cancer endpoints that are going to evaluated for this fall, I assume you're going to have a smoking history on some of the individuals for that. I think that would be

a very important endpoint since 1A1 seems to be a fairly important metabolizing enzyme for atrazine. So I think that's going to be obviously induced in smokers, that'S going to be a real, I think a real important sort of issue related to that. So I assume that information will be known in terms of that.

But to get to Dr. Greenwood's comparison of the human and the rat, there actually is quite a bit known in terms of metabolism of atrazine in terms of its profile in P450 structure. And those data are actually fairly well characterized between humans and rats, so consequently those data should be readily available for PK analysis in terms of uptake, particularly with low level exposures, because, just to give you an example, if you are giving an oral bolus dose, which we've sort of been hearing about, the P450s that metabolize are going to be completely different because in that sense, again I mentioned this earlier, you're CYP3As

are actually going to be more appropriate because they're high KM-based P450s.

So consequently your profile is going to be different, and since those are a little bit higher -- more highly expressed in the GI you might not ever see atrazine actually be absorbed to a certain degree in that sense. However, at low level doses, then a different P450 profile takes place. And, again, that's 1A1, which happens to have a higher KM -- I'm sorry, lower KM, higher affinity for atrazine.

So, again, I think those differences alone will change plasma concentrations, which when -- eventually will change target organ concentrations in terms of your model. I don't think any of that had been sort of evaluated, at least in the literature that I was provided.

So I think those are sort of critical issues in terms of metabolism, and particularly since it seems the metabolites

tend to be not as potent in terms of its interaction than the parent compound. So I think figuring out those sort of issues related to the metabolism, at the gut, the level for the gut, as well as the liver, are critical. And then, again, knowing what we do know, the difference between the rat and the human, you should be able to extrapolate that pretty readily, I would think, between those two.

DR. PORTIER: Dr. Chambers.

DR. CHAMBERS: I think all of these comments have been extremely interesting, and I guess I have the mind set of something that's probably a food contaminant, you're going to get it all as one contaminated apple or something like that. And when Kannan was talking I got to thinking that with the two liters of water that everybody drinks every day, that's distributed pretty well throughout the day as opposed to one big slug.

And so the -- I guess again the PK considerations need to be done of getting that dose over an extended period of time during the day, and it's not going to be comparable very well at all, I don't think, with some of these gavage studies that you're having to compare the endpoints with.

So just keeping all these points into consideration as you do your analysis over the next few months and do the consideration I guess tomorrow on the drinking water frequency and all, a very different exposure pattern with drinking water as opposed to the gavage type studies in the animals.

DR. PORTIER: Dr. Reed.

DR. REED: Yes, as we're talking about PK I was thinking that it's also important what is the dose metrics that you're going to be using to compare the rats and humans for the endpoints of concern. And I'm hearing in some cases area under the curve, in

some cases might be peak, and so it might be, you know, more or less related to the endpoints that you are trying to characterize.

And, you know, according to Penny's suggestion that we do endpoints based on the schematic of things for the mode of action, and it might be different from one step to the other, and that's something to look at.

DR. PORTIER: Any additionally commenters? No? Dr. Lee.

DR. LEE: I just sort of have a question. Reading through this particular question, there's a bunch of it that I don't think has been addressed in this discussion. A lot of good points have been raised, but when we have to answer question 2 tomorrow, we need an idea about what is a duration of interest, or an exposure window of interest. And I'm not sure I've gotten much guidance from this discussion on what sort of exposure windows we should be considering tomorrow.

DR. PORTIER: Dr. Krishnan.

DR. KRISHNAN: Yes, I think most of our consideration was the duration of toxicological concern versus the frequency of monitoring, so that, you know, the frequency for monitoring somehow doesn't give us any data in terms of relevance to the duration of the toxicological effect concern. But based on the toxicokinetic aspects that we heard, or pharmacokinetic aspects we heard, so we tried to answer it on the basis of pharmacokinetics and some of the effects on precursors that have been discussed.

But coming specifically to the exposure window of interest, I think my colleague to the left indicated that there's no specific window that can be defined on the basis of the available data. But I mean when you think of the window of exposure, it depends on the critical tox study though to drive the point of departure assessment. At least that's how I'm looking at it, maybe I

can get some clarification or help from the others.

If the critical window of exposure of the key studies that end up driving this assessment have any specificity of a two-day window during development or something like that, that's how I see a window of susceptibility and window of critical exposure. And based on the discussions that I have seen, it's more of the sustained effect on the specific precursors that would appear to have a key impact on the outcome.

And one of the figures in the document, I thought it was Figure 2, where it showed dose duration relationship as well for effect on the LH surge. I forgot the page number; it was Figure 2, that white paper -- 27. Okay. So that one showed a dose duration relationship for the NOEL from almost a single day to about a six-month duration or so, which essentially relates to the key event on LH surge, which relates to the critical outcome.

So I mean we are kind of -- well, at least from my perspective, from the studies I have heard and seen, I am not getting any other -- well, I don't have any other input. Maybe we'll see if EPA wants to add some clarification to that.

DR. LOWIT: Yes, I'll rephrase maybe what Dr. Lee was thinking in my own words. To go back to one of the things I think Ken had sort of parked, the issue of the duration, the acute versus chronic, I've got open on the white paper, page 29, Table 3, which is the early beginnings of what Penny was mentioning around the temporal concordance table for mode of action analysis.

It shows the currently -- or at least as of two months ago, this is changing fast -- the HPA data for ACTH and cort and progesterone, and some GnRH four days, LH across, one day up to 26 weeks, and some cyclicity data from one day up to 26 weeks. And keeping in mind that we're going to have

some better dose response to fill in a number of those times.

I think it's relevant to think about those durations, everything from that one day cort measure all the way up to the six-month LH endpoint that is the current foundation of the RfD, and pretty anything shorter or in between. It's open for us to pick two days, four days, three and a half, or seven and four-quarters, you know, it's -- four quarters, that eight -- but three-quarters, you know, it's pretty much open to choose whatever makes the most sense biologically.

And what I haven't heard, and I'm hoping we'll hear in the second half of the question, is the dynamic considerations. We've heard a lot of really good points around the kinetic, but what we haven't talked about in this question are the dynamic considerations, the relationship of the key events, the time required to go from changes

in cort to eventually changes in LH, and how you think about those and how that becomes relevant to humans, the translation of the one-day to the four-day to the LH, and then translating that into humans.

Those are the factors that I haven't heard in this conversation that get at I think what Dr. Lee is asking about.

DR. PORTIER: Dr. Krishnan.

DR. REED: Now I recall when I came to the meeting, in reference to your question, Dr. Lee, when I came to the meeting, I said to myself the answer is very simple, monitor every 15 minutes. That's what this table tells me.

(Laughter.)

DR. KRISHNAN: And then as I went along, as the discussions went along yesterday, I think, before the end of the day, I said, I think three or four days or so seems more reasonable. And then I listened to Dr. O'Byrne and then some of our own discussions,

and what I'm convinced is that, you know, the key event essentially relates to a sustained effect and it's all the area under the curve either internally or externally.

That's where I stand right now. And then so the duration is simply you just multiply it with the number of days that you want, and that's where I stand. So I wanted to clarify that.

DR. PORTIER: Dr. Gilliom, and then Dr. O'Byrne.

DR. GILLIOM: So you're getting, you know, a few kind of outside-the-box questions from hydrologists who don't understand anything about, or statisticians, about the toxicology, but we're getting into how you translate the realities of the exposure situation into what's relevant for what ends up being a concentration benchmark.

And one of the questions I'm still a little confused about, and it's kind of in two parts, is one, and this might be an EPA

question, are we still considering the total chlorinated triazine approach, or are we talking only atrazine as a parent compound? And I haven't heard much further discussion of that.

And then second, you know, I think with good reason we're thinking about the actual temporal exposure conditions, both of the variation in the water and the variation of the ingestion that happens. But also what we leave out, and it's probably an impossible question but I've to bring it up, is how comfortable is everyone with the conclusions based on the atrazine functionality models and so forth when you also know it's in the presence of a lot of other contaminants?

And I guess maybe it goes to, more than anything, I want some perspective on that because it really affects how we view the monitoring requirements. If it were important to pick up certain coincident occurrences, then they might have different patterns and we

would have to know how to factor that in to characterizing that.

Now I know it adds probably and impossible level of complexity, but unfortunately it's kind of the reality of what's out there, and that's why I bring it up and look for some reaction.

DR. PORTIER: Dr. O'Byrne. Oh, let Dr. Okana go next?

DR. AKANA: Akana, Okana, doesn't matter.

(Laughter.)

DR. AKANA: Yesterday we saw a slide from Dr. Handa that I thought could be very useful here. He had a slide where he withdrew atrazine so the animal -- seeing it and then you saw the recovery. And I was absolutely, first of all, thrilled to see it was not irreversible, but second, if I remember right, it declined in four days. So an off response might be very helpful in figuring out your window of vulnerability and

duration.

DR. O'BYRNE: In the context of the surge, which seems to be the critical event, it's not like a knee-jerk response. This is a massively complicated neuroendocrine signaling system which starts with a gradual rise in estradiol resulting in cascades of neuropeptide changes in the brain. And this event takes probably a day or two to actually get it to work.

It's triggered by a rise in estradiol, and those levels of estradiol have to be elevated at a critical level for many, many hours, not two or three, we're talking in women it's something like a day. Yes? And in the context of rats, and they have a four-day cycle, so the window is much, much shorter. So that's one of the reasons why I caution against comparing women with rats. I mean it's just outrageous at many, many levels.

(Laughter.)

DR. O'BYRNE: We're talking about

neuroendocrinology here.

DR. PORTIER: I'm glad to hear that. Glad to hear it.

DR. O'BYRNE: So I think in terms of the duration, I think that -- I think it's very difficult for you guys to appreciate the duration in terms of sampling water and things like that. I don't know how you're going to do it, to be honest.

But this is an event that takes a long time to kick start, and in some ways it's astonishing that it takes four days of daily atrazine at phenomenally high doses, and I still don't like that, of 50 milligrams per kilo to knock it out, and then there's no dose dependent nature to that, which, I mean I'm beginning to realize may not be terribly surprising, I mean in terms of, you know, you completely knock it out with 50, 200, 300 and probably 1,000.

It's just either there or it's not. That's what I see in terms of the

ovariectomized estrogen-primed animal, which is a much easier model to deal with because there you've got the response to estradiol is being evaluated. Whereas if you take a spontaneously ovulating rat, the machinery is massively more complicated because you may be interfering with the rise in estradiol, and we don't know the dynamics. If the rise in estradiol is less steep, is not maintained at a certain critical value that may impact markedly.

So the ovariectomized rat given exogenous gonadal steroids to induce this event is a much cleaner model, and that's the one that has been used and shown at three days is necessary to knock it out. So I don't know if that's helpful at all.

DR. PORTIER: Dr. Lowit.

DR. LOWIT: If Ken would let me -- if we can push on that concept a little bit. Let's say theoretically, theoretically, there were some -- there were a data set linking

four days of atrazine to LH to the surge, it got down to something short, in the rat, of something as short as four -- three, four, five days. How would you translate that?

DR. O'BYRNE: It's already at four days.

DR. LOWIT: So how would you translate those rat four days into human days? What sort of scale are we talking about? Are we also on the day scale, are we on the week scale? On a month, because that's a whole cycle?

DR. HORTON: Yes, I need a refresher course. What happens to corticosterone or cortisol in the rat when you treated them with atrazine? I'm going to brain dead at the moment.

DR. COOPER: Yes.

DR. HORTON: Okay.

DR. COOPER: When you dose them it increases.

DR. HORTON: Okay. Cortico -

DR. COOPER: Corticosterone increases.

DR. HORTON: Increases. Okay. Because there's a very large body of data from the `80s and `90s from the laboratory of Neena Schwartz on the effect of steroids on LH and FSH secretion that can give you information on the time course of this, and treatment over the course of five to ten days with corticosteroids prior -- or just treatment with corticosteroids can influence the differential secretion of LH and FSH.

So that might help inform the studies on the set up of atrazine, and also give you some information on the length of time if atrazine setting up the induction of corticosterone, that if you have an effect of atrazine that is inducing the HPA axis, and then you need the exposure of the HPG axis to the steroids to then have an influence on the HPG axis, kind of what the sequence of events would be. So you need to have that kind of

time course.

DR. COOPER: Yes, I'm familiar with Neena Schwartz's work. And you're right, but it's really hard to pin down some of those durations, but that's the information I'm getting, that maybe we have more limitations from the basic literature than I thought.

Back to this dose though, I think I got -- I want to just restate something with the rise in estradiol. In, you know, in a four-day rat estradiol starts to rise around noontime of the day of diestrus 2 and it continues to increase throughout the evening and into proestrus, and that's where you start to have all the dynamic changes that Dr. O'Byrne was referring to.

We've monitored that in intact animals under atrazine. We don't -- again, that's -- a lot of these questions are there and we're trying to get as many answers as we can. And when you dose with atrazine from estrus, diestrus 1, diestrus 2, and proestrus,

those four days, that's the exact same model that was published in Fraites's paper, the doses required to decrease the amplitude of the LH surge at 1800 hours, the effective -- the LOEL in that study was 6.25, the NOEL we got was 3 point whatever dose halves are.

So I think the notion that the estrogen-primed animal, we started out and we continue to work with the estrogen-primed animal. I personally don't really think the estrogen plus progesterone animal is really useful at tox because of the potent signal that P4 gives the pituitary -- I'm sorry, the hypothalamic-pituitary control.

But so the two I like, and these are personal preferences, right, is the estrogen-primed female and the intact animal. Now it takes a tremendous amount of effort to do an intact female period. But because we have unlimited taxpayers' resources, we --

(Laughter.)

DR. COOPER: No, I mean it's -- we

are -- over the -- you know, if you're frugal enough and you're careful enough, you can follow the animals and you can make -- the one thing you have to do if you're following cycles -- if you're doing an intact animal is, number one, and this is really a problem in tox studies, is to make sure your females are cycling. And that's not always the case in a lot of things we get to review.

So we make sure for two weeks that these females show four-day cycles. Then we initiate our treatment and then we follow them, and we kill them -- we have killed them every two hours from 6:00 a.m. in the morning to proestrus throughout -- well, now two hours after dark. So we have now a data set that's quite complete.

And where we're now currently picking apart that rise in estradiol, that rise in progesterone which is just as important in the afternoon, and then where -- now, think about this, we're trying to

evaluate the normal cycle in the animal, but we're dosing them with a compound that we know at some dose is going to disrupt the cycle, so it gets really dicey to make sure that you're dealing with treatment effects as opposed to throwing out affected animals in this kind of thing.

When all that's said and done, we do have a LOEL and a dose response down to a NOEL of 3.125 -- that's the right number -- and then we've looked at estradiol and progesterone, we haven't finished the -- and we're looking at ovulation and all the different things that we can just to make sure that we have that boxed in properly.

So I think the notion that we need -- 3.25 and 6 I think are still probably up there in terms of exposure. I'm not going to say they're low doses, but in terms of the literature anyway that's down there around where our -- the current point of departure is of 1.8, I think is the NOEL; that's pretty

close.

So what we were looking for was a question -- to the answer to this question given that kind of information. It took four days to get there to have an effect on the surge. Is that the kind of exposure that you could say this is -- you know, these things, if you're exposed at some sufficient level for that long, it can be adverse, whether it's through adrenal or direct on the pituitary -- hypothalamic-pituitary axis. So that's one of the questions.

And then the other question is, built into that, is if it's a single-day exposure, and I think I've already this answer, if it's single-day and it's adrenal response, which we may end up with the same dose response but it may be meaningless in terms of an adverse outcome, we can't -- an adverse outcome on adrenal, and I think I've heard that from this panel one time, which is good. I think that's good. But you still

need an adrenal axis.

So I think the question we're getting at is, do you have advice on -- I think I've already heard it, and that one day is probably insufficient right now. So it's not like some other chemicals where we can dose once, wipe out the surge, and it causes problems with ovulation, and it causes problems if the animal becomes pregnant with the embryo development and those kinds of things.

So we do have one day exposure. If it was for a reasonable period for time, should you go -- you know, that's the question that we're asking, or seeking advice on.

DR. O'BYRNE: Okay. Can I ask you a question. Have you tried one, two and three days, or was it just one and four?

DR. COOPER: Not yet.

DR. O'BYRNE: You haven't. You haven't done that.

DR. COOPER: No, we've done one,

two -- oh, yes, we've --

DR. O'BYRNE: You have?

DR. COOPER: No. We've dosed for one or one, two, three, four, but we haven't had the opportunity yet to --

DR. O'BYRNE: And have you looked at the dynamics of the estrogen change in those animals that were dosed for four days?

DR. COOPER: Yes -- have we looked at the entire --

DR. O'BYRNE: Yes.

DR. COOPER: No.

DR. O'BYRNE: On day one, two, three, four?

DR. COOPER: Only from 6:00 a.m. on pro to two hours after lights out, every two hours. And we haven't found a change in that hormone. We've seen changes at nine o'clock after we dosed, because we dose 9:00 a.m. on proestrus as well, and we see changes in progesterone and cort at 10:00.

DR. O'BYRNE: So progesterone is

attenuated?

DR. COOPER: No.

DR. O'BYRNE: It's increased?

DR. COOPER: Yes. At that -- it increases at 10:00 and 11:00, drops back down, then you see the afternoon rise, and it goes right up to the same levels that we see in control, you know, 60 milligrams per -- nanograms per millimeter, somewhere around there.

DR. O'BYRNE: Because of course that progesterone is probably coming from -- it's going to be coming from the adrenals.

DR. COOPER: Yes. And the other thing is that -- and we've looked at uterine growth in those animals and that's one of our key criteria is that you want to make sure that you're uterus, which goes from about 200 milligrams to almost a full gram over that day, it really balloons up, you want to make sure that they're responding because you don't want to have an animal who's not showing that

kind of rise in estradiol because then she wouldn't be proestrus. That was our assumption.

DR. PORTIER: Dr. O'Byrne, is this helping you kind of converge --

DR. O'BYRNE: Yes, I see --

DR. PORTIER: -- on a duration? I mean it's fascinating to watch you guys talk to each other, but I'm trying to figure out if this is helping you converge to this duration question.

DR. O'BYRNE: Well, the -- I mean I think the four-day duration seems a reasonable ballpark. The question of how we relate this to the menstrual cycle, that's a really difficult one to appreciate because I think in the rat you only need a very short period of raised estrogen to trigger this surge, but you need it for days. I think it's something like 30 -- no, no, no, for the raised elevation -- the elevated levels of estrogen in women is a day or two, I believe.

Yes. So I mean that's just massively different.

So if you look at it from that point of view, then you have to -- you know, you can model that you need a much, much longer duration of exposure to interfere with that positive feedback effect of estrogen in women. You've got to couple that with the knowledge that the entire surge generating mechanism in women and primates, higher primates, is just totally different from a rat. You don't even need a brain.

(Laughter.)

DR. O'BYRNE: You don't even need a brain if you're a woman to have a surge. It's an absolute truth.

DR. COOPER: Well, that's --

DR. O'BYRNE: The data is there --

DR. COOPER: -- I'm sorry --

DR. O'BYRNE: The data is there --

DR. COOPER: I understand Nobel, sorry --

DR. O'BYRNE: The data is that it would be estrogen -- the estradiol -- the positive feedback effect of estradiol is the pituitary phenomenon.

DR. COOPER: It's pituitary, in the monkeys, Nobel's work, but I think there's more than sufficient evidence to show that a lot of chemicals that work essentially to inhibitor or impair the pulsatile release of GnRH and release of LH in humans. And I know the pulse frequency is important, and it's -- just as well in humans.

But I think the -- back to the duration question, I think we could scale that up and scale it down, and I think that's part of the issue. You're right.

DR. PORTIER: Dr. Schlenk.

DR. SCHLENK: Yes, just a simple question. I mean do you know from your ex vivo studies what tissue concentration you need, say in the adrenal glands, to get a cortisol release?

DR. COOPER: No, not now.

CHAIR HEERINGA: Steve Heeringa. I think you've started to address what was going through my mind, and statistically, statistically, the most pathological case in terms of these chemographs and exposures are these short term surges we see, and we had a couple of examples, the Missouri raw water thing that we see where you have several spikes in atrazine concentration in the raw water supply, and they last for about two or three days.

And in terms of thinking about the coincidence of sort of the sampling frequency we'll be talking tomorrow, and the concerns you're talking about, it's the first of June, Thursday, it rains, Sunday morning I make coffee and I get a surge of atrazine in my community water supply, if it happens that fast. I make the same coffee Monday morning and Tuesday morning. By Tuesday morning it's gone, it's back down to a lower level.

I think what is the impact of that maybe for me, or for women in the household, et cetera. I think that's the key sort of sampling issue that we need to get at. I think if you're saying that it's sustained concentrations at certain levels over a period of time, the statistical issues will become a lot easier.

But it's the -- and most of the chemographs, and Nelson and others could confirm this, that we saw, even in raw water, there maybe are two peaks, two or three peaks per year that are real spikes, and most of them are much more uniform flat. And I assume in finished water it even looks flatter yet, so. But I think you were getting at that when you were talking about this before.

DR. PORTIER: Dr. Williams.

DR. WILLIAMS: I just have one comment about the female menstrual cycle. It does take a much longer time than the rat to actually create a menstrual cycle, but it's

not clear that it would take much longer or potentially any longer at all to disrupt that.

So if you have an atrazine spike that's the day before or two days before you're going to have an LH surge, just for that one day, could that be disruptive? Based on all the central effects we've been talking about is that enough to disrupt? It's certainly not enough to create a menstrual cycle, obviously you need the days and the feedback and so forth, but how long does disruption take?

DR. O'BYRNE: I think you're perhaps asking the wrong person. I mean there are experts in the US who know a great deal more about the control of the menstrual cycle in women. But I would be very surprised if you perturbed the follicular phase by -- during that two-week period you perturbed it on one particular day, I'd be amazed if it impacted on that follicular phase. I'd be absolutely astonished. It just wouldn't make

sense to me.

I'm just thinking of my time when I was working with monkeys, you really do have to make a major stressor to perturb the menstrual cycle. I don't think one event would make a jot of difference. And this was in the context of restraining Rhesus monkeys in primate chairs. It didn't disturb the cycle. I think --

DR. PORTIER: Dr. Horton.

DR. O'BYRNE: -- it's quite -- I think it's quite robust.

DR. HORTON: This is where I think when we finally see epidemiological data and kind of the absence of data on the developmental issues comes into play because it's in development that neuroendocrine systems and neurotransmitter systems are much more sensitive, and where exposure for transient -- or exposure to chemicals for transient periods of time can have serious long term effects.

And so if you take a hit for a day or two at just the right time of development, and unfortunately because we're talking about humans, we don't have experimental data on it, and we never will have experimental data, and you really can't extract it very well, even from epidemiological data, but certainly looking at rodent data, the work that's been done with steroids, you know, treating them at the right stage of the prenatal period for one day with the right steroid can have major developmental effects.

Most of this work was done looking at the effects of sexual differentiation, but we're now finding that different critical periods exist for, say, treating with androgens that results in changes in metabolism later in life resulting in the recapitulation of factors like metabolic syndrome.

So I think taking into consideration the possibility that exposure to

these pulses or periods for over a few days at the wrong time of fetal development might be important. Also what we haven't seen any data on, and I don't know if this will addressed at some other SAP, might be the -- whether there are any data on impacts on in vitro fertilization, early pregnancy, loss. If Dr. Lowit would like to comment on whether any of that is going to be addressed.

DR. PORTIER: Actually, before we get to that, I think the hour's getting late. We long ago wiped out my desire to have the hydrology discussion.

What I wanted to suggest is if we can capture some of these unanswered questions, you know, the kind of things that we've been -- we haven't had any answers, but we've had some questions that I think will help EPA to refocus how you think about duration and, you know, some of the issues of women's menstrual cycle, the level needed to impact it, at least in higher primates, the

key reproductive and what we know about, if I understood, steroidal impacts on key reproductive and developmental points.

You know, if we can kind for ask the right questions, that'll at least help the EPA staff kind of begin to formulate the answer. I don't think we quite have gotten to your -- the answer to your questions, but I don't think Dr. Lowit really expected an answer to the question.

If that's agreeable? Any final kind of concluding remarks on this point? Dr. Krishnan.

DR. KRISHNAN: Could it be a confusing remark?

DR. PORTIER: Huh?

DR. KRISHNAN: Could it be a confusing remark rather than a concluding one?

(Laughter.)

DR. PORTIER: That would be an additional confusing remark.

DR. KRISHNAN: Okay. I just want

to -- before we -- at least in mind I just wanted to get a clarification. So as we see these various precursors that are being modified, you know, which is listed in Table 3 where we've got, you know, a change occurring in 15 minutes in cort level, or over a longer period the effect on LH surge and so on, does that -- well, the way I see it is that the monitoring frequency then has to match with this, with essentially with each one of these key event or precursor, whichever you pick, so your frequency will have to match that somehow.

But then not each one of these precursors are going to make it through the risk assessment. Like, you know, maybe the cort level is not going to be used in determining the PoD or something. So I think that's where -- that's what has to get into this equation to determine whether we need to ask this question about these various precursors and the frequency of monitoring.

Because if not all of these get to -- get into the process of deriving -- or driving the PoD, then I don't see that being a critical factor in this consideration. I'll leave it at that.

DR. PORTIER: Thank you.

I'm going to actually leave this question open just in case somebody comes up with a brilliant idea in the morning. There's no reason to close it. But I think at this point we're going to break for the day and begin again tomorrow morning at 8:30 again with kind of just a recapitulation of this, and then we'll go into the discussion.

I'd like the hydrology people to hang around for a few minutes here after the meeting so we can talk strategy for tomorrow.

Thank you very much.

(Whereupon, at 4:51 p.m., the meeting was adjourned, to reconvene at 8:30 a.m., Thursday, April 29, 2010.)

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